REPORT OF THE CHIEF OF THE BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY AGRICULTURAL RESEARCH ADMINISTRATION

1951

UNITED STATES DEPARTMENT OF AGRICULTURE

Report of the Chief of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, 1951

UNITED STATES DEPARTMENT OF AGRICULTURE,
Washington, D. C., October 5, 1951.

Dr. P. V. CARDON,
Agricultural Research Administrator.

DEAR DR. CARDON: I present herewith the report of the Bureau of Agricultural and Industrial Chemistry for the fiscal year ended June 30, 1951.

Sincerely,

G. E. HILBERT,
Chief.

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INTRODUCTION

The report for the fiscal year 1951 covers 96 items grouped under 21 titles that indicate in a general way the fields of research receiving attention from the Bureau of Agricultural and Industrial Chemistry. It is impossible, within the limits of an annual report, to tell what progress has been made in each of the many research projects on which work has been done, so the report is confined to what are considered some of the more important accomplishments of the year and to commercial applications of research results previously reported.

The Bureau's research activities are concerned with either the solution of scientific and technological problems involved in the utilization of agricultural products and byproducts, or the acquisition by basic research of new scientific knowledge that might bear upon the utility of agricultural materials for various purposes. Although the items in the report deal with the more striking achievements, they do not necessarily reflect the proportions of time and effort devoted to applied research and to fundamental research. Most of them deal with projects in applied research, a type that usually yields tangible results more quickly than does fundamental research.

A little less than one-fourth of the items deal with new additions to fundamental scientific knowledge, some of which were sought because of immediate need in connection with practical utilization problems. Ten of them cover industrial applications of new research results or of results mentioned in previous reports. About two-thirds of the items cover recent progress in applied-research projects on utilization of agricultural products and byproducts, in 22 of which the work has advanced through pilot-plant experiments, demonstrations in industrial plants, or laboratory experiments in sufficient number, or on a sufficiently large scale, to be ready for application in industry or in commercial testing or research laboratories. One item deals with the results of economic research, incidental to technological research on utilization.

Industrial application of research results

A new machine developed at the Southern Regional Research Laboratory for opening and fluffing matted cotton from the bale, preparatory to cleaning and carding, is now in commercial production and use. Three manufacturers of textile and cotton-ginning equipment are making it, and six additional licenses have been granted to machinery manufacturers and mills for use of the Government-owned patent.

An improved technical grade of oleic acid is being produced in tank-car quantities from animal fats for industrial chemical uses. Several large processors of fats and fat acids are applying a process developed by laboratory and pilot-plant investigations at the Eastern Regional Research Laboratory.

A commercial firm which undertook the production of allyl starch, after it was developed by the Eastern Laboratory, found that the ever-increasing demand for the material for use in lacquers and other products required several increases in its production facilities during the past 4 years. Allyl starch is used for furniture finishes, interior wood coatings, and heat-setting adhesives, and as a vehicle in printing inks.

Dilinoleic acid, a dimeric fat acid prepared from soybean oil several years ago, by the Northern Regional Research Laboratory, and shown to be useful for upgrading drying oils and in making synthetic rubbers, rubber substitutes, polyamide resins, and viscosity-index improvers for lubricants, is now in commercial production by a new process developed by an industrial firm which cooperated in the earlier work.

At least two commercial firms in the United States have produced vitamin-B_{12} concentrate for use in mixed feeds by an entirely new fermentation process developed at the Northern Laboratory. Other firms have made successful preliminary fermentations by using the cultures of *Streptomyces olivaceous* and directions for their use supplied by the Laboratory and are preparing equipment for large-scale production of the vitamin.

Permanent glass color standards to represent the limits of six grades of extracted honey have been developed by the Eastern Laboratory in collaboration with the Production and Marketing Administration. The standards, which have been made official by the Department, together with the equipment for using them, are commercially available. They are being used by Government inspection services and by buyers and producers of honey.

Tobacco shade cloth treated with lead chromate to retard damage by sunlight has been produced commercially, after its value was proved by the Southern Laboratory in cooperative service tests extending over three seasons.

Norconidendrin, produced for the first time at the Southern Laboratory from conidendrin, a constituent of waste liquor from the pulping of western hemlock wood, and shown there to have value as an oil stabilizer, has been made available on a limited scale by a commercial firm.

In cooperation with a filter manufacturer, the Naval Stores Research Division modified a commercial self-cleaning filter to adapt it for use in cleaning pine gum preparatory to steam distillation. The first commercial installation of the new self-cleaning filter in a gum-processing plant has been made.

As a service to manufacturers of dried citrus pulp for feed, who needed to know how the addition of citrus molasses would affect the keeping quality of the product, the Citrus Products Station at Winter Haven, Fla., demonstrated that the addition of molasses did not introduce any serious storage problem. At relative atmospheric humidities of 70 percent or less, citrus pulp with or without added citrus molasses can be stored without spoiling, but, at humidities of 80 percent and over, the product will absorb enough moisture to mold and spoil. The Station also developed a method for estimating the soluble-solids content of citrus feeds, which is simple, rapid, and accurate enough for routine control work. The method is being used by feed mills.

In addition to its achievements of previous years that have found new or expanded commercial use during the year, the Bureau has—

Helped to prove that good newsprint and improved grades of certain specialty papers can be made by blending fine pulp from wheat straws with eastern, Lake States, and certain western wood pulps.

Helped to demonstrate that the wood veneer commonly used in wire-bound shipping containers can be replaced by thin, compact panels of strawboard protected on exposed edges by thin strips of metal.

Prepared high-density, full-flavor apple-juice and grape-juice concentrates (about sevenfold) that kept in good condition for a year in ordinary cold storage.

Prepared experimental lots of frozen apple-juice concentrate of three kinds for a consumer-preference test, and later 40,000 6-ounce cans of frozen fourfold concentrate of one kind for a market test, to guide the apple industry of Washington State in the production of frozen apple-juice concentrate.

Helped to demonstrate the technical practicability of recovering sugars and other soluble solids from pear-cannery waste in the form of a clear colorless juice that is useful as a sirup base in canning pears, thereby accentuating the pear flavor and conserving sugar.

Showed that dehydrated vegetables of greatly improved storage stability can be prepared by a combination of practices designed to prevent or retard nonenzymic browning and oxidation.

Demonstrated a distinct advantage from scalding sweetcorn on the cob before cutting it off for freezing preservation.

Found that the development of pink centers in frozen brussels sprouts can be prevented by first heating sufficiently to inactivate the enzyme peroxidase, as indicated by a prescribed test.

Prepared a chart that gives cucumber-pickle packers the exact proportions of sugar and vinegar required to put up sweet pickles so they will keep safely.

Verified by experiments on a commercial scale the role of enzymes in the spoilage of salt-stock cucumbers, and devised control methods.

Developed a procedure for making accurate counts of the spores of anaerobic toxin-forming and putrefactive bacteria in nonacid foods after incubation for only 24 to 46 hours in a special medium, whereas incubation for many weeks may be required with other methods.

Developed a simple, rapid, reproducible method for indirectly determining the water content of fruits and other foods by measuring the quantity of potassium dichromate required to oxidize the nonaqueous substance.

Showed by actual service tests that cottonseed-protein glues are satisfactory for bonding plywood intended primarily for interior use in dry climates.

Developed a formula for a mixture, composed mainly of abundant domestic starches, that can replace the sweetpotato starch formerly used in making nonfreezing electric dry cells but no longer available commercially.

Found that the highest allowable moisture content of unhulled tung fruits for safe storage before milling is 12.4 percent in equilibrium with air at 75 percent relative humidity.

Developed and published a method for preparing copper chlorophyllin (a medicinal derivative of chlorophyll) from leaf meal made by dehydrating broccoli trimmings.

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Helped to develop improved candies in disk form suitable for packing in cans for the Armed Forces.

Showed that vegetable-tanned insole leathers can be retanned with commercially available basic aluminum acetate solution to increase serviceability of military insole leathers.

Developed practical methods for extracting, purifying, and concentrating tanning material from canaigre roots, and showed that canaigre extracts can be used alone to produce sole leather or in blends to produce lighter-weight leathers.

Found vinyl esters and ethers of long-chain fat acids to be useful for increasing water resistance of vinyl-type plastics and reducing their tendency toward embrittlement by low temperatures and migration of plasticizer.

Developed and thoroughly tested a continuous steam still, having a throughput of 5 tons an hour, for producing turpentine and rosin from cleaned pine gum.

Produced from gum turpentine the new compound pinane hydroperoxide, which was found superior to cumene hydroperoxide now used as a catalyst in making synthetic rubber.

Translating research results into industrial use

Usually attempts to solve a chemical or technological problem relating to industrial utilization of an agricultural commodity begin with exploratory research in one of the four regional research laboratories. If this results in a process that seems to have industrial possibilities, a pilot plant for testing the process may be designed, constructed, and operated in the pilot-plant wing of the laboratory. Or if reports of the preliminary results made at scientific meetings, or published in technical or trade journals, arouse the interest of industrial firms or associations, one of them may undertake pilot-plant work with collaboration of the Bureau. This is what happened in the case of allyl starch. Information may be exchanged between project leaders and representatives of industrial firms in correspondence, in formal conferences, or informally in visits at the laboratory or at industrial plants.

If pilot-plant work has been done by the Bureau, cooperation with an industrial firm or association may be arranged for further development work in a larger pilot plant, or even in an industrial plant when the process is ready for final testing. If the exploratory laboratory work results in a new product that has industrial possibilities, substantial quantities of the product may be prepared and samples supplied without cost to possible users for evaluation. This was done with dilinoleic acid, pinane hydroperoxide, allyl starch, and deresinated guayule rubber. Some cottonseed-oil mills and tung-oil mills have tried out, at their own expense, new practices advocated by the Bureau to determine practicability, and the operators of some gum-turpentine plants have made ther facilities and labor available for trying out new developments in gum cleaning, continuous steam distillation of cleaned gum, and removal of acid from the gum collected from acid-stimulated trees. Many such examples could be cited.

There are various other ways in which industry can participate in pertinent research. One is by supplying materials for experimental work. Arrangements can also be made for an industrial firm or a

non-Government research organization to conduct a special phase of a research project with its own personnel and equipment under the direction of a Bureau project leader. Such arrangements are made through a research contract. Less extensive cooperation may be arranged by a cooperative agreement, under which each party contributes to the cost. Research fellows, selected and supported by associations representing major segments of industry, are welcomed in Bureau laboratories to conduct research under direction of Bureau scientists on problems where mutual interest can be defined.

The story of the development of a new machine for opening and fluffing matted cotton from the bale typifies the chain of circumstances which contribute to the successful translation of laboratory research on better utilization of agricultural commodities into industrial practice. The prototype of the present opener was designed as a feeding unit for a manufacturing process that prepared cotton lint for use in making gun cotton during World War II. The potentialities of the feeder for civilian textile applications were early recognized, and at the end of the war a project was proposed for the development of an opening machine employing the basic principles of the feeder. This project was reviewed and approved by the Cotton Advisory Committee of the Department and work was started on the construction of pilot-plant models of the opener.

Several years later, a large processor of cotton for surgical uses became interested in the efficient and speedy manner in which this machine prepared cotton for further processing and arranged to have a large-scale unit built by a machine manufacturer. The value of this first commercial model has been proved in actual practice and it has been determined that the opener will pay for itself many times during the first year of its operation because of the large savings that result from its use. Several other cotton processors, impressed by the performance of the opener, have installed the machines in their textile mills.

Publications and patents

Further information on the Bureau's work is available in the official publications that appeared during the year. Bureau employees contributed 115 articles to the 1950–51 Yearbook of Agriculture, entitled "Crops in Peace and War," which dealt in a general way with the utilization of agricultural materials. Other printed and processed publications issued during the year numbered 428. Most of them were research papers published in non-Government scientific and technical journals. Usually a limited number of reprints of such papers are bought for distribution to seriously interested individuals and firms as long as the supply lasts.

Information on the Bureau's research projects under way the first part of the fiscal year 1951 is given also in the three-volume report, Research and Related Services in the United States Department of Agriculture, by the Agricultural Research Administrator to a special subcommittee of the Committee on Agriculture, House of Representatives, Eighty-first Congress, second session, December 21, 1950.

Information on newly developed processes and products is given in the specifications of the 83 patents granted during the year to employees of the Bureau.

COTTON FIBER AND ITS PRODUCTS

New Cotton Opener Used by Textile Mills

A commercial-size machine developed at the Southern Regional Research Laboratory for opening and fluffing the matted lumps of cotton from the bale before cleaning and carding was described in last year's report. In September 1950 details of the opening machine were released to the industry. As a result, the opener is now in commercial production and use. Three manufacturers of textile and cotton-ginning equipment are making it, and six additional licenses have been granted to machinery manufacturers and mills for use of the Government-owned patent. Four openers are in operation in the mills. One firm which bought two of the machines finds that their use has resulted in large savings. It reported: "We are sure it will pay for itself many times during the first year of operation." The savings will lower the manufacturing costs of textile products. It is estimated that savings up to $1 per bale on the lower grades of cottons processed are being obtained.

The new opener, with exceptionally high production (1 ton per hour) for its small size (requiring only 50 square feet of floor space), is a great aid in cleaning mechanically harvested cottons. The high speed of the opener does not significantly increase the formation of neps, and the grade and strength of yarns are unaffected. In addition to its value as an aid in cleaning, the machine reduces the amount of spinnable fiber removed with the trash by cleaning equipment.

Methods Found for Reducing Neps and Improving Quality of Cotton Goods

Neps are minute, tangled masses of fibers formed in cotton during processing. They detract from the appearance of cotton fabrics and lower their quality where appearance is of first importance. Such defects are especially undesirable in fabrics that are to be dyed, for the neps dye to shades different from those of the body of the fabric.

Methods for substantially reducing the formation of neps during manufacture were discovered in research by the Textile School of the North Carolina State College of Agriculture and Engineering under a Research and Marketing Act contract with the U. S. Department of Agriculture. The work is being supervised by the Southern Regional Laboratory. Nep formation is influenced by fiber maturity, fineness, and length, as well as by excessive manipulation of the fibers during the opening, picking, and carding processes. It was found that neps can be reduced through careful selection of the raw stock and by making adjustments in the settings and speeds of the opening, picking, and carding equipment. The greatest improvement can be made at the card by applying methods which distribute the cotton in thinner and more even sheets on the cylinder and by closer settings between working parts of the card to prevent rolling and nepping of the fibers. Reducing the number of opening processes and reducing the beater speeds, or adjusting the rate of cotton feed to obtain milder treatment of the fibers, also will reduce nep formation.

Certain recommended measures have been adopted by a number of cotton mills, and substantial improvements in yarn quality have been reported.

Luster Characteristics of American Cottons Surveyed

American cottons can be selected effectively for the manufacture of end products in which maximum luster is desired as a result of work on improving the luster of cotton conducted by the Harris Research Laboratories in Washington, D. C., under a Research and Marketing Act contract with the U. S. Department of Agriculture. The work is being supervised by the Southern Laboratory.

For this research it was evident that the first step must be development of an accurate and dependable means of measuring or characterizing luster in textiles. Because the lustrous appearance of a yarn or fabric depends chiefly on the manner in which light is reflected from its surface, the method developed measures mechanically the light-reflecting properties of the cotton textile. It utilizes instruments and techniques usually employed in estimating gloss or reflectance of non-cotton materials, such as ceramics. The mechanically determined values of luster in textiles correlate well with the results of visual judgment in a wide range of materials.

A survey was made in which this method was applied to American cottons, embracing 103 varieties grown in 25 locations. The measurements showed that significant differences existed between varieties, but that each variety tended to hold similar rank from location to location in relation to other varieties grown at the same location. Correlation between fiber properties and yarn luster was found to be greatest for the X-ray angle and fiber strength, properties that relate to internal structure, and to be less marked for fiber length and fineness.

The new equipment, techniques, and knowledge developed in this study should aid further cotton research and breeding work and facilitate the selection of cottons for specific end uses.

New High-Strength Cotton Evaluated

A working plan for the evaluation of new and superior varieties of cotton has been developed through the coordinated efforts of such groups as the National Cotton Council and State and Federal agencies, including the Southern Regional Laboratory. The Southern Laboratory's part in the evaluation program includes determinations of special physical properties of fibers, mechanical processing characteristics, responses to chemical treatments including mercerizing and dyeing, and small-scale tests of the products in a limited number of selected end uses. A system of this kind can be used to speed the evaluation of essential properties and performance of the new varieties while they are still available in only small lots. The information gained will be of value to cotton breeders, and will also assist mills in evaluating some of the new cottons as they become available in larger quantities.

As a participant in this program, the Southern Laboratory investigated the properties of one of the new high-strength cottons developed by the Bureau of Plant Industry, Soils, and Agricultural Engineering, namely Hopi Acala 50, a variety that seems particularly suitable for growing in California. To determine whether the high strength had been obtained at the expense of other desirable properties, such as resistance to damage by flexing, tire cord manufactured from the new cotton was evaluated. Wheel tests were run on tires

made from Hopi Acala 50 cord and from a cord produced from a variety of comparable staple length, Stoneville 2B. The Hopi Acala cotton cord showed satisfactory flex life in tires as compared to that of the control. Evaluation of the mercerization properties of the new variety in yarn form showed that by mercerization it attained a higher degree of luster than Acala 4–42, the variety now grown in California, but there was no significant gain in strength by Hopi Acala over the gain by Acala 4–42.

The techniques developed in connection with the evaluation of Hopi Acala 50 will be applied in the evaluation of small lots of interspecies cottons, when experimental lots of these cottons are obtainable.

Survey Shows Importance of Cotton Waste

A recent study of the production and utilization of cotton waste in the United States points the way for improving the efficiency of cotton-textile processing and utilizing more effectively the different types of waste produced.

This study, conducted jointly by the Southern Regional Laboratory and the National Cotton Council, showed that about 15 percent of all cotton consumed in the United States becomes cotton waste in the process of manufacture in textile mills. About 70 percent of the total cotton waste is fiber, the remainder consisting of motes, bits of leaf and stem, dust, and other foreign matter. In 1948 United States mills produced 642 million pounds of cotton waste having a value at the mill of $71,000,000.

The results of this study have provided a greater appreciation and knowledge of the size and nature of the waste industry, the factors influencing waste markets, methods for controlling waste, and opportunities for enhancing the value of cotton waste through research.

The large number of requests received for copies of this report—about 1,000 in 8 months—is evidence that this type of information is interesting and useful to a large part of the textile industry.

Reuse Value Shown for Cotton Fertilizer Bags

A recent development in the struggle of cotton-bag manufacturers to maintain cotton's competitive position has been the practice of supplying cotton flour and feed bags dyed and printed in attractive colors and patterns. Because their excellent reuse value was readily apparent, such bags immediately became popular, and this popularity has greatly helped in retaining an important outlet for cotton in the face of competition of lower-cost paper bags.

The success of this venture suggested to manufacturers the possibility of applying the same plan to the sale of cotton fertilizer bags. There was some fear, however, that cotton bags might be harmed by chemical action of the fertilizer with which they were filled, leading to loss of fabric strength or to fading of the colors used, or perhaps to both. After some debate on this point, the problem was referred by the Textile Bag Manufacturers Association to the Southern Regional Laboratory for investigation.

Representative samples of dyed and printed bag sheeting, as well as a quantity of commercial 5–10–5 fertilizer, were supplied by a member of the association. The laboratory filled several sets of small

test bags with fertilizer and stored them under different atmospheric conditions. One set was kept under conditions approximating those that would normally prevail in a warehouse in New Orleans; another was placed in a hot, dry atmosphere; and a third was stored in excessively warm, moist air. At regular intervals bags of each color and pattern were withdrawn for examination. At the end of a year there were no significant changes under any of the conditions employed. Experts in matching dyes could see no difference in color. Strength losses, by standard laboratory tests, were insignificant. Thus an indication that colored or printed cotton bags can be packed with fertilizer without appreciable damage to the color of the fabric was provided.

On the basis of results of the tests, the Textile Bag Manufacturers Association and the National Cotton Council have proceeded with their plans to encourage the use of dyed or printed cotton fertilizer bags. Their efforts, according to recent reports, have been attended with considerable success.

Weather-Resistant Tobacco Shade Cloth Produced Commercially

Field trials by Florida tobacco growers of a weather-resistant lead-chromate treatment for cotton shade cloth applied experimentally by the Southern Regional Laboratory were described in a previous report. The success of the trials has resulted in the commercial production of such treated fabric, which is now available in any desired quantity. This year one tobacco grower in northern Florida bought enough yardage of the treated cloth to cover a 10-acre field.

Increased durability of the treated cloth is responsible for the interest of tobacco growers. Samples first tested in 1948 have been used for three full seasons as top cover, and are being used a fourth season as wall cover. In contrast, untreated cloth is so rapidly degraded by the action of sunlight that it serves only one season as top cover and one as wall cover. The increasing price of cotton materials made shade cloth such an important item in the tobacco farmer's production costs that the demand arose for protective treatments to prolong its useful life. Commercial application of a lead-chromate treatment that effectively protects the cloth from sunlight is not a complicated operation, and the expense involved is small in comparison with the saving in replacement costs of untreated fabric.

Effects of Mechanical Processing on Fiber Properties Measured

Knowledge of the relations between fiber properties and yarn and fabric qualities is important in research to improve the usefulness of cotton products. Recently interest has arisen in the significance of the changes that may take place in cotton fibers as they are processed from raw stock into yarn. Any damage to the fiber during processing would be carried over into the products and affect their utility.

Investigations have been made in this field at the Southern Regional Laboratory. Last year it was reported that studies of the effects of mechanical processing on the individual fiber strength, elongation at breaking point, and viscosity showed that the only significant change in processed cottons was decreased elasticity. In further experiments, ginned stock from seven commercial American cottons

was processed through the yarn stage. Again only the elongation and the modulus of elasticity, which depends upon elongation and is a measure of toughness, were affected; the elongation was consistently reduced, and the modulus increased. There was no evidence of damage to the fiber during spinning. This proof that spinning changes only the elongation properties makes it possible to use yarns rather than the unprocessed raw stock in evaluations of the various chemical treatments developed at the Southern Laboratory. Therefore the yarns prepared in the processing experiments have now been subjected to these chemical treatments for evaluation of their effects on physical properties of cotton yarns.

In an exploratory investigation under an RMA project, knowledge was obtained on the changes in the mechanical properties of cotton fibers that result from heating them in the presence of moisture. Fibers were heated for various periods in atmospheres of different humidities at 110°, 138°, and 162° C. Generally the loss of strength was greater as the moisture content of the atmosphere increased, but an atmosphere of 100 percent moisture—live steam—was considerably less destructive, probably because of the absence of oxygen. Data of this kind will be applicable to processes in which yarns or fabrics are subjected to changes in the surrounding atmosphere, as in dyeing and the application of size to warp yarns.

Advance Made in Testing Cotton Textiles

Although the cotton industry has for many years supplied the world with durable and satisfactory textiles in almost every essential civilian and military market, certain advances towards greater efficiency of operation and improved quality of products are needed. This need is heightened by the present state of national emergency. Cotton growers, merchants, processors, and research workers will be aided in their task of effecting the required improvements by having better testing methods and new information on the significance and the application of the tests. Improved testing methods and equipment could speed the work of the cotton breeder and research worker, lower the cost of processing control, provide new information on the relation between fiber properties and yarn and fabric performance, give improved products by indicating the best end uses for specific lots of cotton, and conserve supplies of cotton.

It is believed that the following advances in textile testing made by the Southern Laboratory the past year will have practical value for many laboratories and mills.

Improved X-ray technique for determining crystallite orientation

Fiber strength is closely associated with the orientation of the cellulose crystallites in the cotton fiber. For many years scientists have used this crystallite orientation, or the "X-ray angle," as an index of fiber strength. Such an index is particularly useful in selecting varieties of cotton for use as research standards and in guiding the development of new cotton varieties. The measurement of orientation, unlike the usual mechanical strength tests, may be carried out on a very small tuft of fibers, even those from a single seed. A more rapid method of determining the X-ray angle is provided by the

development at the Southern Laboratory of a new rotating specimen mount for use with an X-ray spectrometer. The new procedure eliminates expensive photographic processes that are required with other techniques, and, since it traces the X-ray curves automatically, dispenses with the usual time-consuming densitometer measurements.

Conditioning samples before testing

There has been considerable uncertainty as to whether cotton yarns and cords to be tested for breaking strength and elongation must be brought to equilibrium moisture content under the standard conditions (65 percent relative humidity at 70° F.) from a lower or a higher moisture content in order to obtain reliable results. This point has now been settled by experiments at the Southern Laboratory. Preconditioning of cotton yarns and cords before their exposure to the standard atmosphere for tests of breaking strength and elongation did not offer any practical advantage, and the expense of preconditioning all samples to approach moisture equilibrium from either the high side or the low side does not seem to be warranted.

Permeability of cotton fabrics

Although practical tests for measuring the permeability of cotton fabrics to air and water have long been available, much still remains to be learned about the underlying factors involved in achieving the desired fabric properties. It has been found that the pore-size distribution—arrangement and size of void spaces in fabrics—is one of the determining factors. This knowledge was gained by use of a special technique and an instrument devised for determining the amount of mercury that enters the dry fabric when immersed in mercury under pressures ranging from 1 to 600 pounds a square inch.

The technique was applied to learn the pore-size distribution in 18 cotton fabrics of selected closely woven constructions and to correlate the pore-size distribution with their known properties. Differences in the distribution were found to be related to differences in the maturity of fibers and tightness of weave and also to changes produced in the fabrics by scouring and finishing. Knowledge of this correlation between fabric properties and pore-size distribution helps to clarify problems encountered in research on developing airtight and watertight cotton fabrics. The method will be useful also for correlating the pore-size distribution with thermal conductivity and other properties that are dependent upon the void spaces in the fabric.

Mathematical expression for fiber roundness

One of several bases for predicting the behavior of cotton fibers in certain end uses is knowledge of their cross-sectional shape. All cotton fibers are twisted tubes, but the tubes can vary in shape from ribbonlike flatness to almost complete roundness. It is known that the roundness of fibers has an imporant influence on end-use characteristics of the fibers or final textile. Fiber shape, for example, is a good measure of maturity and affects luster, resilience, suitability for mercerization, breaking strength, and dyeing qualities But one difficulty in establishing definite correlations has been the lack of a sound quantitative method for designating shape by means of a simple function that can be used as a numerical index.

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Now a simple mathematical expression and an instrument have been devised in analytical and cotton-fiber research at the Southern Laboratory to evaluate the circularity of fiber cross sections and to measure the quantities defining the shape. The new technique, which has been used in connection with research to develop better water-resistant cotton fabrics, has general application in all investigations involving a correlation of shape and fiber properties. The instrument and the index in this way will be useful tools in gaining a broader understanding of the relations between individual fiber properties and the useful properties of yarns and fabrics. Several research agencies, including the National Bureau of Standards, have shown interest in using the new technique.

OILSEEDS AND THEIR PRODUCTS

New Chemical—Dilinoleic Acid—Produced Commercially From Seed Oils

Dilinoleic acid (commonly called dimer acid), which is a dibasic, unsaturated fat acid derived from soybean and some other seed oils, is now in commercial production by a new process. Manufacture of the new chemical, which may find widespread industrial use, is largely the result of pioneering research on dimeric fat acids by the Northern Regional Laboratory and of continued cooperative research that has been carried on by the Northern Laboratory and certain chemical industries.

Dimer acid has been studied extensively at the Northern Laboratory to determine its utility in chemical industries. In a series of technical papers on the preparation, properties, and utilization of dimer acid, issued by the Laboratory during the years 1942 to 1949, information was given on how industry could use the acid for upgrading drying oils and for manufacturing rubber substitutes, polyamide resins, viscosity-index improvers for lubricants, and synthetic rubbers. The polyamide resin Norelac, which was developed at the Northern Laboratory from dimeric fat acids obtained from soybean oil, was discussed in the 1943 and 1944 reports.

During World War II industrial companies undertook the production of rubber substitutes and polyamide resins from dimer acid, and commercial utilization of these products was achieved. Need for rubber substitutes became less urgent with the success of the synthetic-rubber program, but commercial manufacture of polyamide resins has continued because of the demand for this material as a moisture-proofing and heat-sealing agent, particularly for glassine paper wrappers for candy bars and other food products. Recently it appeared there would be more extended use of dimer acids in drying oils and protective coatings because of a serious reduction in the quantity of tung oil available for domestic use.

In consequence of the increasing interest in dimer acid, so largely stimulated by the work of the Northern Laboratory, one company investigated better methods of producing the acid. A successful process was developed, and dimer acid is now available in commercial quantities.

Linolenic Acid Causes Undesirable Flavors in Soybean Oil

Studies supported by RMA at the Northern Regional Laboratory show that linolenic acid is a flavor-unstable component of soybean oil. The experiments discussed in last year's report demonstrated that the flavors peculiar to aged soybean oil could be imparted to otherwise flavor-stable cottonseed oil by introducing linolenic acid into the cottonseed oil.

Further confirmation of the major part that linolenic acid plays in causing the undesirable flavors has now been obtained. Some of the volatile flavoring substances isolated and identified by the University of Pittsburgh (in RMA contract research) have been found to be formed by storing linolenic acid esters in the presence of oxygen. Moreover, studies of the oxidation of linolenic acid esters have demonstrated that it proceeds in a different manner than the oxidation of cottonseed oil esters. Cleavage of the linolenic acid molecules to give volatile flavoring substances occurs at the very early stages of storage. This is in contrast to the much later occurrence of similar chemical reactions in esters of flavor-stable oils. The apparent difference in reaction mechanism for linolenic acid esters may account for the difference in flavor stability of soybean and cottonseed oils.

Acid From Corn Stabilizes Flavor of Soybean Oil

Phytic acid constitutes the most recent and promising discovery at the Northern Regional Laboratory of compounds that stabilize the flavor of soybean oil. This acid, in combination with calcium and magnesium, is present in many foods, particularly the cereal grains. It is available from corn wet-milling plants, where it is a component of the steep water. Like citric acid and sorbitol, both of which have been advocated as oil stabilizers by the Northern Laboratory and widely accepted by the soybean-oil industry, phytic acid stabilizes soybean oil by virtue of its ability to inactivate naturally occurring and contaminating metals. When these metals (principally iron and copper) are uncombined, they start or accelerate the oxidative deterioration of the oil and speed the development of undesirable flavors.

Phytic acid has a distinct advantage over citric acid, sorbitol, and related compounds. When phytic acid is added at the beginning of the deodorization step in refining soybean oil, its capacity to counteract the injurious effect of added metals is evident, not only during deodorization, but throughout the subsequent storage life of the oil. Pilot-plant studies and semicommercial trials indicate that phytic acid, added to the deodorizer at the rate of 1.5 to 3.0 ounces per ton of oil, gives deodorized oils of initially high flavor quality and of improved stability during storage.

Consistency of Soybean Lecithin Modified

Modification of the consistency of commercial soybean lecithin has been accomplished by a chemical treatment developed under an RMA project at the Northern Regional Laboratory. Reaction of lecithin with isocyanates has given products that range in physical form from

viscous liquids through soft and hard waxes to hard, brittle, resinlike solids, depending on the type and amount of isocyanate employed.

A waxy, semibrittle product was prepared on a pilot-plant scale by reacting lecithin with phenyl isocyanate. In contrast to the unmodified lecithin, it formed with comparative ease water suspensions that showed reduced surface tension and improved stability toward microbial action. Upon request, this product has been supplied for evaluation to potential users, such as manufacturers of printing inks and floor waxes.

"Soy Flour" Improved for Use in Greece

The use of "soy flour" in bread has been urged for European countries during the past few years because of the need for more protein in diets. The use of soy powder in Europe presents problems different from those in the United States. Europeans use long-extraction wheat flours of rather low protein content, and much of their bread is made without sugar and shortening. In Greece, for example, bakers use flour of 90-percent extraction and never any sugar or shortening. As Greek bakeries are small and their equipment is crude, it is not to be expected that practices of modern bakeries in the United States can be adopted and applied.

A representative of the Economic Cooperation Administration's mission in Greece reported to members of the staff of the Northern Regional Laboratory the difficulties which Greek bakeries had encountered in using soy powder in bread. The results were so unsatisfactory that in one year less than 10 perecnt of their allotment of soy powder was used. Research under an RMA project at the Northern Laboratory indicated the possibility of overcoming some of the objections to the use of soy powder and of making possible a corresponding improvement in the Greeks' diet.

A series of experiments at the Northern Laboratory showed that addition of oxidizing agents, such as potassium bromate, was necessary when fortifying high-extraction flours with soy powder. When 5 percent of soy powder was used without oxidizing agents, the bread was inferior. The volume of the loaf, which normally is small in comparison with that of bread of equal weight in this country, was reduced by a third; thus the bread became unappetizing even to people ordinarily accustomed to heavy bread. It was found that, on addition of the correct amount of potassium bromate, the loaf volume became normal and other undesirable characteristics disappeared. However, it was evident that because of the crude working conditions and the small size of their bakeries, the Greek bakers could not use the oxidizing agents by themselves. Hence it was suggested that the correct amount of potassium bromate be added to the soy powder at the time of its manufacture.

Representatives of the Economic Cooperation Administration and of the Production and Marketing Administration were informed of the results of the Laboratory's work and of the proposal for improving the situation. They agreed to the proposal and immediately made arrangements for the purchase by the Economic Cooperation Administration of a trial shipment of bromated soy powder to Greece. Through the splendid cooperation of the "soy-flour" industry of the

United States, the powder was manufactured with addition of the specified proportion of oxidizing agent. An initial shipment of 1,000 tons has been sent to Greece.

Improvements Made in Hydraulic Pressing of Cottonseed

Hydraulic pressing, for many years the only process used to recover oil from cottonseed kernels, has lost ground to screw pressing, solvent extraction, and recently to the combination of forepressing and solvent extraction; however it is still used for more than half the cottonseed crushed each year. This method probably will continue to be important for a long time, especially for smaller mills that cannot justify the increased investment required to convert their operations to the newer techniques. For this reason improvements in the hydraulic-pressing method are being sought by the Tennessee Engineering Experiment Station under an RMA research contract with the Agricultural Research Administration, supervised by the Southern Regional Laboratory.

One of the major objections that processors have had to hydraulic pressing has been the amount of oil left in the press cake. Under the best operating conditions the press cake contains between 5 and 6 percent oil. The present investigations, conducted on a laboratory scale, have been directed specifically toward the solution of this problem.

If full advantage can be taken, on a practical scale, of the information provided in these experiments, there is a possibility that residual oil in hydraulic-press cake may be reduced about 1 percent below the average values. This would amount to an increase in cottonseed-oil production of about 10 pounds per ton of seed crushed. Not only would this add to the total dollar value of the products of hydraulic pressing, but it would also provide more oil that might be urgently needed under emergency conditions.

The investigation has included a study of different conditions for each step of the process, including variations in temperature and time of cooking, moisture content of the meats during cooking and pressing, the amount of pressure and rate at which pressure is applied, and the time and temperature of pressing.

Improvements in oil extraction on a laboratory scale were obtained by raising the temperature and lowering the rate and total amount of pressure. It was observed, however, that such changes did not bring about entirely independent effects, indicating that a combination of optimum conditions must be found to obtain the maximum benefits. Further work has been planned for this purpose.

Improved Process Found for Removing Color From Refined Cottonseed Oil

Some cottonseed oils, particularly those produced by screw pressing and refined by conventional methods, are not of acceptable color and quality for use in the manufacture of shortening. Such oils are often subjected to a second refining. Even after rerefining they still may not meet specifications for a shortening oil with respect to color.

Experiments at the Southern Regional Laboratory have demonstrated that the conventional rerefining process can be modified to

produce acceptable products from these off-color oils. Essentially the modified process comprises the use of increased concentrations of caustic soda solution, and very high rates of agitation and shear in mixing the oil and alkali, within certain specified temperature limits. The changes from the conventional process, while simple, are radical, as the conditions found to be most effective for removing the color were previously believed to be deleterious to the oil and represented unacceptable refinery practice.

The optimum conditions under which the new process should be operated were established by systematically investigating the five variables affecting the process—concentration of the caustic soda solution, total amount of caustic soda used, speed of agitation, duration of agitation, and temperature.

Reduction in color of refined cottonseed oil was found to increase as the rate of agitation and shear of rerefining increased, up to an extremely high rate, beyond which further increases had little or no effect. About 0.2 percent of caustic soda (on weight of oil), used in the form of a 14- to 24-percent solution, agitation for 5 to 10 minutes, and a maximum temperature of 150° F. were found to produce maximum reduction in color. The color of the oils rerefined by the high-shear method usually was not more than half as dark as the color that would be obtained in the same oils by conventional rerefining. This proportional difference in color was also observed in the oils after bleaching. The high-shear rerefining method removed the acidic gossypol pigments of cottonseed oil more effectively than did conventional rerefining. In addition, the new method removed a high percentage of unidentified red pigments unaffected in conventional rerefining.

Aside from the fact that it requires some special mixing equipment and consumes more power than does conventional rerefining, the new process has no known disadvantage. In it conventional materials are employed, the oil requires no special treatment before or after rerefining, and rerefining losses are the same as with the conventional process.

Antioxidants Tested as Preservatives for Cottonseed Oil

During recent years a number of chemical compounds whose properties indicate they would be useful in stabilizing fats and oils against oxidative rancidification have become available. Most of them are phenols differing widely in chemical structure—some relatively simple, others complex. There has been considerable information on which to base the use of some in certain fields, but usually not enough to be a reliable guide to their effectiveness as antioxidants. The information on others, however, was meager, and data for evaluating the over-all usefulness of these new products were quite inadequate.

As part of its program of research to improve the utilization of oils from southern-grown oilseeds, the Southern Regional Laboratory in 1951 conducted an investigation aimed specifically at providing data for comparing the effectiveness of different antioxidants in stabilizing cottonseed oil against rancidity.

In previous research the Laboratory had demonstrated that cottonseed oil, when carefully processed and handled, has a high inherent stability toward oxidative rancidification because of certain natural substances (tocopherols) that are present. Hydrogenation, the process

used in preparing oils for use in shortenings and margarines, further improves the natural keeping quality. Nevertheless, additional improvements are desirable if they can be attained.

The Laboratory tested 13 antioxidants for their ability to improve the keeping quality of a finished edible cottonseed oil and the same oil hydrogenated to shortening consistency. For comparison, the same antioxidants were added to prime steam lard, a product that is relatively low in natural antioxidants. As might be expected, they were much more effective in stabilizing the keeping quality of the lard than they were for either of the oils. Propyl gallate was found to be the best of the antioxidants tested for both hydrogenated and unhydrogenated cottonseed oil. While less effective than propyl gallate, nordihydroguaiaretic acid and norconidendrin were found to be good antioxidants. Most of the other 10 antioxidants tested gave little or no increased protection to cottonseed oils, although they were effective with lard, even in small concentrations.

This study completed a thorough investigation in which much new and valuable information on the stability of cottonseed oil has been provided by the Southern Laboratory in recent years. Of particular significance are data explaining the mechanism by which fats and oils absorb oxygen and become rancid. Working first with the simple unsaturated fatty acid ester, methyl oleate, and later with cottonseed oil, the reaction of atmospheric oxygen with these substances was found to produce hydroperoxides, which on decomposition produced various saturated and unsaturated aldehydes that impart rancid odors and flavors to the fat or oil. This knowledge, obtained in work on cottonseed oil, applies also to peanut oil, and has been extremely valuable in developing the additional knowledge that is now available to improve the keeping quality of fats and oils in general.

Norconidendrin—New Oil Stabilizer—Made Commercially

Norconidendrin, a new antioxidant made from a compound that is recovered from the waste liquor produced during the pulping of western hemlock wood, recently has been made available on a limited scale by a commercial concern. This development is based on work carried out at the Southern Regional Laboratory in recent years and renewed in 1950 in cooperation with the manufacturer of the new product.

Evidence showing that norconidendrin has many potential uses has been obtained. For example, it is very effective in protecting fats and oils against rancidification. Small amounts added to cottonseed oil, peanut oil, and lard greatly increase the shelf life of these products. Other important possibilities include the stabilization of candies containing butter and of wax-paper coatings. Because norconidendrin appears to have many possibilities in the food and food-packaging fields, studies were started recently at the Western Regional Laboratory to learn whether or not the product can be used safely in edible products.

Other applications of norconidendrin have been made in the stabilization of unsaturated hydrocarbon distillates, lubricating oils, certain unsaturated insecticides, and oil-well drilling muds. Recent tests have shown that this product is highly effective also in retarding the deterioration of certain butadiene copolymers, as well as in the stabilization of vinyl-type monomers. Furthermore, it is reported to be effective in

preventing the aging and discoloration of compounded rubber articles.

Many of the older commercial antioxidants are made from benzene and other chemicals that, because of the present emergency, are in short supply. Hence the use of norconidendrin, which is made from byproduct liquors of pulp mills, has the advantage of conserving critical chemicals.

The success of norconidendrin illustrates vividly the application of chemistry to enhance the natural properties of a product for greater usefulness. While searching for more effective antioxidants to prolong the shelf life of vegetable oils, Southern Laboratory chemists recognized a similarity between the chemical structures of compounds that had been used for this purpose previously and conidendrin, a substance known to be present in the wood of certain trees, including hemlock. Conidendrin itself was found to have only a slight antioxidant effect when added to edible oils and fats. However, by altering the chemical structure of conidendrin, the chemists produced an effective new antioxidant, which they named norconidendrin.

Prior to this development, conidendrin had been a laboratory curiosity, although the possibilities for recovering it in large quantities as a byproduct of pulping western hemlock wood by the sulfite process on the west coast were known. Tests have shown that from 10 to 15 pounds of conidendrin can be recovered at moderate cost from the byproduct liquor of 1 ton of western hemlock pulp. Conversion of the conidendrin to norconidendrin is somewhat more costly, but at present the product sells for one-eighth the price of some of its competitors. Hence its use for certain purposes might effect a considerable saving in some of the industries depending upon antioxidants to insure the output of high-quality products.

Advances Made in Coordinated Research on Cottonseed Meal as Feed

Preliminary steps taken by the Southern Regional Laboratory to guide the planning of research to improve the nutritional value of cottonseed meal were described in the annual report for 1950. At that time information had been obtained justifying the inauguration of a broad program of investigations under an RMA project to develop methods by which meals of higher nutritive value could be produced for more extensive use in the mixed-feed industry. Some of the results of this work are reported below.

Experimental meals suitable for hog and chick feeds

Investigations in cooperation with agricultural experiment stations and the cottonseed industry are pointing the way to unrestricted utilization of cottonseed meal as the protein supplement in hog and poultry rations, as well as in feeds for cattle. Preliminary results indicate that experimental cottonseed meals produced in commercial quantities by a modification of the screw-press method can be fed to hogs in concentrations as high as 43 percent of the total diet and to chicks in concentrations up to 70 percent of the diet without any harmful effect and with good support of growth. Heretofore recommendations for the use of cottonseed meal in these feeds called for concentrations of 10 percent or less for hogs and sometimes as little as 5 percent for chickens. These restrictions can be lifted as soon as meals equal in dietary value to the experimental meals are produced regularly by the industry.

Nutritive value related to chemical analysis and processing history

A definite relationship has been observed between processing conditions and the quality of cottonseed meal. Surprisingly, though, there is about as much variation among different meals produced by the same process as there is among meals produced by different processes. This was demonstrated in 1950 when samples of about 100 commercial cottonseed meals, produced by screw pressing, hydraulic pressing, and solvent extraction, were analyzed for free gossypol, nitrogen solubility, and other factors that might influence their nutritive value and were later fed to chicks and hogs. The extreme variability observed in the chemical analyses was confirmed by results of the feeding tests.

Even meals described as identical by present trading rules, which base the characterization largely on nitrogen content and color, were widely different in both chemical analysis and feeding results. Some of the meals were far superior sources of protein than the usual characterizations indicated. As a result, nutrition investigators now insist upon information about the conditions under whch meals are processed and their chemical analyses, in addition to the usual market description.

New approach to research on cottonseed meal as feed

With more and more investigators giving serious consideration to the effect of processing variables on the value of the product, an entirely new approach to research on the utilization of cottonseed meal is under way. Heretofore nutrition investigators have had the complete responsibility of procuring the meals, having them analyzed, and determining their nutritive value. Under such an arrangement it was difficult to give sufficient attention to the effect of processing variables.

Accordingly the Southern Regional Laboratory, in cooperation with the National Cottonseed Products Association, developed a new improved arrangement for nutrition research that provides for the cooperation of all parties involved from the production of the meal to its evaluation as feed. Under this arrangement, cottonseed meals are produced in commercial mills under conditions specified by the Southern Laboratory. The meals are analyzed and characterized at the Laboratory and then are furnished to nutrition investigators in State, Federal, and commercial laboratories. The arrangement makes it possible to correlate the results of feeding tests with the chemical analyses and processing history of the meals. Systematic changes can be made in processing conditions so as to study the effect to better advantage. In the last 3 years, 40 tons of experimentally produced cottonseed meals have been supplied for nutrition research under this program.

This coordinated program of research on cottonseed meals, with emphasis on processing history as related to nutrition, is paying off with information that promises to greatly increase the production of high-quality cottonseed meal. As the need for protein supplements for feeds and foods is unusually great in emergency periods, the acquisition of new knowledge on cottonseed meal is now particularly timely.

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Usefulness of Cottonseed Meal for Plywood Glues Established

The Southern Regional Laboratory extended the tests reported last year on the use of glues made with protein obtained from cottonseed meal from a laboratory to an actual-service basis. The results confirm previous indications that cottonseed-meal glues, when they become available commercially, may be used in the plywood industry. Added to the information on hand, the service tests provide all the data needed by industry in judging the potential usefulness of the new cottonseed product.

The service tests were of two kinds—"interior severe" and "exterior." Both were conducted under failure-accelerating conditions to speed development of the needed information. Plywood panels bonded with the glue were first subjected to the interior test, during which they were kept for 48 hours under warm, humid conditions (80° F. and 80 to 85 percent relative humidity) that were more severe than would ordinarily be encountered in indoor use. When this exposure was completed, the panels were allowed to come to moisture equilibrium in an atmosphere of 32 percent relative humidity at temperatures between 80° and 85° F. This was repeated for five cycles, and the reduction in bond strength noted. In the exterior test, samples were immersed in water at 75° F. for 48 hours and then dried. This cycle was repeated until the glue failed to hold the panels together.

The results showed that cottonseed-meal glues are entirely satisfactory for bonding plywood that is intended primarily for interior use in regions where the average relative humidity is low. Where the humidity is high, cottonseed-meal glues might not produce a sufficiently permanent bond for satisfactory use.

In its earlier experiments on the development of cottonseed-meal glues, the Southern Laboratory found that solvent-extracted meals produced with a minimum of heat gave stronger bonds than glues prepared from cottonseed meals made by other processes. Meals for experimental use were produced under ideal conditions in the Laboratory's pilot plant. When the usefulness of cottonseed-meal glue was definitely established, the studies were carried a step further to determine whether or not commercially produced solvent-extracted meal could be used. It was found to be satisfactory. As the number of plants producing solvent-extracted meals is increasing, there is no longer any question about the availability of raw material for the manufacture of cottonseed-meal glue on a commercial scale.

Control of Roasting Conditions Improves Quality of Peanut Butter

In continued research to determine the exact effect of processing conditions on the quality of peanut butter, the Southern Regional Laboratory, under an RMA project, has prepared butters from peanuts roasted under widely different conditions and has evaluated them both chemically and by tasting after storage at 80° F. for as long as 21 months.

These evaluations, together with the results of previous laboratory and pilot-plant studies, show definitely that careful control of roasting conditions is necessary for the production of peanut butter of optimum flavor and good keeping quality, and, furthermore, that the range in desirable roasting conditions is rather narrow.

New data also support previous observations that the oil contained in peanut butter is relatively stable to oxidative rancidity. Oils obtained from fresh peanut butters were found to have low peroxide values and long keeping times. Although some reduction in keeping times of oils extracted from peanut butters under storage was found to occur during the first 3 months of storage, the keeping times of these oils were still relatively long. No appreciable differences were found between the keeping times of oils from butters after storage for 3 months and those from butters after extended storage.

Taste-panel tests indicated a preference for medium-roasted butters stabilized with hydrogenated peanut oil for the prevention of oil separation, the average scores for such products being higher than those given to lighter-colored butters. In general, peanut butters stored at 80° F. and examined periodically by the panel were found to retain acceptable odor and flavor for as long as a year, after which the products developed objectionable flavors before any appreciable oxidative rancidity could be detected in the extracted oils. Butters made from the heavier-roasted peanuts tended to receive unsatisfactory flavor ratings at an earlier age than butters made from the lighter- and medium-roasted peanuts.

This information is of value to the peanut-butter industry because it makes possible the selection of conditions of processing that will produce butters having a high consumer rating and long shelf life. It is also of interest to the Quartermaster Corps because its application will assure the production of peanut foods of high quality.

Safe Moisture Content Found for Storing Tung Fruit Before Milling

Investigations at the United States Tung Oil Laboratory in Bogalusa, La., provided new information on the water-absorbing and water-holding properties of tung fruit that is useful for solving one of the major problems in utilizing the domestic tung crop. This problem is proper drying of the fruit and seeds to insure safe storage and efficient milling.

When the tung fruit falls from the tree it contains about 65 percent moisture and will heat, mold, and sprout if stored before it has dried to about 25-percent moisture. Even under favorable weather conditions, natural drying takes several weeks; sometimes in wet seasons the fruit never dries on the ground sufficiently to permit storage. Artificial drying of the whole fruit, on the other hand, is not practical.

Because the hulls contain more than half the total moisture in the whole fruit and have no value as a source of oil, their removal before drying or storage is desirable to decrease the heat required for drying and also reduce the space needed for storage. However, hulling followed by the usual storage procedure has some disadvantages that are discouraging to growers. It has been found, for example, that seeds broken in the hulling operation quickly spoil if stored in their normal moist condition. They develop free fatty acids rapidly and sometimes heat spontaneously. But research has also shown that tung fruit, seeds, and kernels tend to have their moisture contents balanced with that of the surrounding atmosphere. This means, of course, that knowledge of the equilibrium moisture contents of these products at different relative humidities can play an important part in their drying and storage; it was this knowledge that the Tung Oil Laboratory sought in its investigations.

It was found that tung fruit, seeds, and kernels in contact with air at 75 percent relative humidity (average for the tung-growing area) have equilibrium moisture contents of about 12.4, 8.2, and 6.1 percent, respectively. Consequently, if the products are dried to these moisture contents, they can be stored safely and processed efficiently. It is particularly significant that the hulls can be removed and the seeds dried to 8 percent moisture content with assurance that they will not spoil in storage.

In recent work the Laboratory has extended these investigations to determine safe storage conditions for tung hulls and press cake, which frequently spoil as a result of spontaneous heating. The results show that these products, too, can be stored safely if they are first dried to moisture contents in equilibrium with air at 75 percent relative humidity. These equilibrium moisture contents are 17.1 percent for tung hulls and 10.3 percent for press cake.

Fat Derivatives for Flexible Coatings Made From Monoglycerides

For a long time there has been a need in the food industry for an edible, highly flexible, nongreasy fat coating material to replace paraffin and other nonfat coatings now used. In general, flexibility and lack of greasiness have been mutually exclusive properties of fats. A nongreasy fat is usually hard and brittle; a flexible fat is usually soft and oily.

Recently, however, the Southern Regional Laboratory, in work under an RMA project, developed flexible and nongreasy fat derivatives that can be prepared in a fairly simple manner. Monostearin, a monoglyceride prepared from glycerin and completely hydrogenated cottonseed oil or similar fat, is allowed to react with acetic anhydride, an inexpensive commercial chemical that unites with water to form acetic acid. The monostearin and acetic anhydride are heated together for about 1 hour at a temperature of about 230° F. The product of this reaction, acetostearin, needs only to be washed with water and in some cases deodorized prior to use.

Various acetostearins having melting points ranging from 80° to 140° F. can be produced. The melting point of any particular acetostearin depends on the grade of monostearin used and the degree to which it is permitted to react with the acetic anhydride. Generally, such products do not have an exact melting temperature, but melt within a temperature interval of 2° to 5° Fahrenheit.

Below their melting points and down to room temperature most acetostearin products are nongreasy and flexible. Below room temperature, most of the experimentally prepared acetostearins could be stretched more than 800 percent without breaking. Even the least flexible acetostearin product stretched six times as much as did paraffin wax (melting point, 125° F.). At freezing temperatures the acetostearin products are still flexible.

Although no known facts indicate that these flexible fats are not edible and digestible, they are being carefully evaluated in this respect by several laboratories before being recommended for use in foods. Their possible use as coatings for cheese, dressed meats and poultry, ice-cream bars, and other food products is being explored, along with other interesting possibilities in nonfood fields.

Efforts Made to Improve Storage and Processing of Rice Bran for Oil

Interest in the production of rice-bran oil as a byproduct of the rice-milling industry has grown rapidly in the past few years, largely as a result of technical information made available by the Southern Regional Laboratory. The oil can be extracted readily and economically, and, if extracted from fresh bran, its quality is excellent. However, several problems remain to be solved before all the available bran can be utilized as a source of oil. Their solution was the objective of further work on rice bran under an RMA project.

Safer storage procedures

One of the problems stems from the fact that rice bran does not keep well between the time it is removed from the grain and the time is is processed for oil. This has resulted in a serious storage problem because facilities are inadequate for immediate extraction of all the bran available.

When rice bran is stored free fatty acids form rapidly, so the oil deteriorates in a very short time. To solve this problem, it is necessary first to understand the cause. For this reason the Southern Laboratory has been conducting a thorough investigation of the factors that contribute to the formation of free fatty acids in rice bran during storage. Evidence has been obtained that the rate at which the free fatty acids form depends on the moisture content of the bran. However, the biological factors responsible for the deterioration of rice-bran oil occur in the bran itself or in the micro-organisms found in the bran. During the past year, therefore, an investigation was made of the micro-organisms in bran as related to moisture content and to the formation of free fatty acids.

Although the investigation is not complete, because information is not yet available on the effect of the enzyme systems of the bran itself, information has been obtained that should be of practical value to the rice-bran-oil industry in developing means of storing the bran without deterioration.

For example, experiments on sterile bran inoculated with selected strains of mold and bacteria, demonstrated that these agents can cause the development of free fatty acids in the bran oil and, therefore, must be controlled in order to completely prevent deterioration during storage. It was observed further that moisture hastens the growth of both molds and bacteria. Molds grow fastest when the relative humidity is about 80 percent; bacteria, at 100 percent.

The obvious conclusion is that rice bran should be stored in as dry a condition as possible. While the moderate heating normally used to dry rice bran was found to destroy very few of the micro-organisms present, and apparently did not affect the enzyme systems at all, such heating did remove the water that permitted hydrolytic reactions involved in the formation of free fatty acids. Thus, if bran dried in this manner could remain dry, deterioration would be prevented. However, it was observed that whenever the dried bran was exposed to the high humidities prevailing in the southern rice-growing areas it absorbed moisture, permitting the natural systems of micro-organisms in the bran and oil to promote deterioration.

Heating the bran with steam under pressure killed all micro-organisms and removed the natural tendency for the bran to deteriorate.

Regardless of the moisture content, free fatty acids will not form in bran that has been thus sterilized and maintained in a sterile condition. Sterilization followed by dehydration, therefore, would seem to be a method for complete control of deterioration during the period when rice bran must be stored prior to solvent extraction. Even if the bran absorbs some moisture during this period, the onset of deterioration will be slow.

Better refining techniques

In another line of research the Southern Laboratory obtained information to improve techniques for refining rice-bran oil. In the past there have been difficulties, especially when the oils were high in free fatty acids, as they often were when the bran had been stored for considerable time prior to extraction. An experimental procedure that involves steam stripping of the oils seems promising as a solution to this problem. The crude rice-bran oil is degummed and dewaxed in the usual manner; then it is treated with steam before the usual refining step, in which an alkali is used.

In pilot-plant experiments steam stripping reduced the free fatty acid content of rice-bran oil from about 9 percent to less than 1 percent and lowered refining losses enough to yield 8.5 percent more oil. Additional studies are under way to determine the best conditions for practical application of this procedure.

Basic information for processors

To assist the industry in considering the economic aspects of rice-bran-oil production, the Southern Laboratory has made a study to obtain more definite information on the amount of oil that might be expected from different lots of bran. Samples of bran from eight commercial varieties of rice grown in Texas, Louisiana, and Arkansas have been analyzed to determine the influence of variety and environment on the oil content. Purified bran (comprising the true pericarp and germ fractions) contained from 18 to 25 percent oil, depending more on the variety of rice than on the locality where grown. The amount of oil in commercial rice bran is lower because the milling operation removes some of the inner starchy part of the grain (endosperm) along with the bran. Variations in milling, as well as in physical condition of the grain, affect the amount of oil.

GRAIN CROPS AND RESIDUES

New Knowledge on Wheat Interests Milling Industry

Flour millers know that some wheats do not mill as easily as others. In the Great Plains region, wheat is bought on the basis of class, such as Hard Red Winter or Hard Red Spring, so varieties that cause trouble in milling are not separated and are not easily recognized. In the Pacific Northwest, however, wheat is bought on the basis of both class and variety. There it is well known that flour production is slowed down when the varieties Rex and Brevor are milled. That is a serious matter when full production is needed for national defense.

Because poor-milling wheats give low flour yields and decrease mill capacity, the milling quality of wheat is of great importance. Although environmental factors (weather and soil conditions and

farming practices) affect milling quality to some extent, certain wheat varieties are known to be consistently good or poor in milling behavior. The varieties having undesirable milling properties, however, may be those best suited agronomically to certain areas. Consequently, farmers in these areas must choose between growing a productive but poor-milling variety difficult to market or a less productive but better milling variety for which there is a ready market. The development of new varieties having both good milling and good agronomic properties could be speeded if poor-milling hybrids could be detected and eliminated at an early stage in breeding experiments, when only small samples are available, but at present new selections must be propagated for several years to provide enough grain for milling tests. Methods for early determination of milling and baking properties are urgently needed when new strains of diseases become prevalent and rapid development of new resistant varieties is required.

A coordinated effort to identify the factors responsible for differences in milling characteristics is being made by the Western Wheat Quality Laboratory of the Bureau of Plant Industry, Soils, and Agricultural Engineering at Pullman, Wash., and the Western and Northern Regional Laboratories of this Bureau. The Western Wheat Quality Laboratory has collected samples of known variety and history, obtained milling data, and supplied these data and portions of the samples to the other two laboratories. The Western Regional Laboratory is studying the relation of differences in chemical composition to milling behavior; the Northern Laboratory is determining whether structural differences of importance in the milling operation can be found by microscopic examination of kernel sections.

As reported last year, work done in the Western Laboratory suggested that a crude fat-to-crude fiber ratio above 1.0 in a whole wheat indicates the type of poor-milling behavior that is characteristic of the Rex variety. Further analyses on 1950-crop samples gave results consistent with the previous results. The significance of the fat-to-fiber ratio was suggested by the possibility that a relatively high fat content contributed to the known difficulty in sifting Rex flour and mill streams by causing the flour particles to clump.

Milling behavior of wheats is too complex, however, to depend upon any one factor. Because the fat-to-fiber ratio does not differentiate between certain varieties (Baart, Orfed, Brevor) that are intermediate in milling properties and the best milling varieties (Elgin, Hymar), other factors must be involved.

The endosperm of poor-milling wheats has been reported to fracture in random manner upon application of pressure, whereas that of good-milling wheats fractures along cell-wall lines. To determine where in the milling process these or other differences in endosperm properties might affect milling behavior, the bran of Elgin and Brevor samples was removed by a sulfuric acid-water treatment. Then the milling behavior of the debranned samples was determined on a micro-mill by the Western Wheat Quality Laboratory. Certain characteristic differences—in release of middlings and rate of reduction of middlings—between the varieties were still present. Consequently these differences in milling properties must be attributable to differences in endosperm, rather than in bran, of these varieties. In

exploratory experiments, analyses for various hemicellulose fractions that are characteristically associated with plant-cell walls have been made by the Western Regional Laboratory. The results suggest that the amounts of certain pentosan fractions may be useful in predicting endosperm milling properties: further investigation may provide more precise methods for indicating milling behavior by chemical examination of small samples of wheat.

Thickness of the bran-forming seed coats, the outer part of the wheat kernel, was previously thought to affect ease of milling and amount of flour obtained. But work recently completed at the Northern Regional Laboratory under an RMA project has shown that this is not true, at least for Pacific Northwest wheat varieties. Total thickness of bran was measured for seven varieties, including Rex and Brevor, which have poor milling quality, and Elgin, which mills excellently. No relationship was found between bran thickness and either the ease of milling or the yield of flour. Likewise thickness of each of the different layers that make up the bran was measured for the seven varieties. Again no relation to milling behavior was found.

When the results of these studies were reported to flour millers, much interest was shown. They corrected a false idea about the relation of bran thickness to ease of milling and yield of flour. In addition, the systematic study of kernel structure and composition was recognized as laying a foundation for developments that should assure rapid milling of wheat to give the high production of flour so essential to national defense. The results found so far show that factors responsible for milling quality must reside within the inner part of the kernel. Studies are therefore in progress to find out which of the structural and chemical factors inside the kernel determine milling quality.

Wheat-Straw Pulp Found Suitable for Fine Papers and Newsprint

Interest in fine straw pulp has increased in the United States and in Europe, South America, and the Orient. The interest in this country is mainly due to the advance in cost of wood pulps and the shortage of newsprint, although interest on the Pacific coast is partly due to the fact that no hardwood species are available for producing the short-fibered pulps that are needed for blending with coniferous wood pulps. The mechano-chemical process of the Northern Regional Laboratory, which was discussed in the 1949 report, is receiving special consideration for producing such pulp from straw.

Several attempts were made to interest some pulp-producing company in making 50 to 100 tons of fine straw pulp in order that a number of interested paper companies might make machine runs on blends of fine straw pulp with wood pulps to produce improved grades of specialty papers. Regular production demands made it impossible to arouse interest in the proposal, so arangements were made to carry out the study at the Forest Products Laboratory in Madison, Wis., on a small scale. Two cooperative series of papermill runs were made on that Laboratory's experimental paper machine.

The first run was for the special benefit of a west coast paper company interested in the use of fine straw pulp as a blend with certain western wood pulps to produce improved grades of specialty papers.

For this purpose Pacific coast white wheat straw was pulped at the Northern Laboratory by the mechano-chemical process and shipped to the Forest Products Laboratory. Part of the straw pulp was bleached before being used. A series of papers were made on the experimental paper machine, either unbleached or bleached straw pulp being used to the extent of about 20 percent of the total pulp, and wood pulps prepared by the west coast company being used for the remainder. As it was necessary to make comparisons with all-wood-pulp papers, machine runs were made with each of the wood pulps alone. This study showed that all of the papers containing straw had improved formation and surface characteristics, even though the straw had not been processed so as to give optimum physical properties. In some cases the strength of the straw-containing papers was increased over the strength of the all-wood-pulp papers. This preliminary study was so successful that the west coast paper manufacturer is installing a small paper machine in a new pilot plant and expects to experiment further with straw pulp.

In the second series of cooperative paper-making experiments at the Forest Products Laboratory, pulp prepared from Illinois straw at the Northern Laboratory was used as a blend with eastern and Lake States wood pulps. From all-wood pulp, and from the blends of the same wood pulp with straw pulp, the following types of papers were made on the experimental paper machine: Magazine-book paper base for coating, soda-book paper, writing bond paper, waxing paper, greaseproof-glassine paper, kraft bag-type paper, and newsprint.

In producing these papers the straw pulps were processed so as to obtain their optimum strength and other physical properties. Depending on the particular grade of paper, from 20 to 80 percent of straw was used in the blends. In all cases addition of processed straw pulp resulted in better formation and surface characteristics, in higher strength, and in slightly lower opacity and bulk, but with better body in the paper. Particularly outstanding, as compared with the all-wood papers, were soda-book, writing bond, and waxing papers containing straw. The magazine-book and the waxing papers were converted by paper companies into finished coated magazine-book paper and into waxed paper, respectively. The magazine-book paper was judged equal to the standard, all-wood-pulp, coated magazine-book paper; the waxed paper was considered an improvement over the all-wood-pulp sheet, particularly because of greater transparency and better formation, which gave better printability and eye appeal.

For some time the Northern Laboratory has believed that newsprint could be manufactured from de-inked waste newsprint and a certain proportion of fine bleached straw pulp. This blend should produce a sheet having the softness, opacity, ink reception, and running characteristics of standard newsprint. An opportunity to prove this belief was afforded at the time of the previously mentioned experimental paper runs. For this purpose unprinted waste newsprint was repulped and mixed with 20 percent bleached straw pulp. The paper proved to be superior to standard newsprint in color, strength, and surface characteristics. It seems practical, therefore, to give consideration to relieving the shortage of newsprint by producing fine straw pulp and blending this with de-inked, repulped waste newsprint.

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Boxboard From Straw Found Useful in Wire-Bound Shipping Containers

About 1.5 billion square feet of low-grade wood veneer, from one-eighth to one-quarter inch thick, is used annually in wire-bound shipping containers. Such containers require only about half as much wood as do the heavier all-wood cases, and their use results not only in a saving of wood but also in lower freight costs. But uses of such wood veneer have increased rapidly over the past few years, and this has raised the price and created a shortage in the supply of veneer for shipping containers. To remedy this situation, a leading manufacturer of wire-bound-box-making machinery undertook the development from annually produced plant materials of board products that might replace the wood veneer in wire-bound boxes. The Northern Regional Laboratory cooperated in this development, since it appeared that an outlet for about 800,000 tons of straw per year might result if the research proved successful.

This cooperative development work has come to a successful conclusion after 4 years of joint effort. More than 400 3- by 3-foot boards were made from straw at the Northern Laboratory. Panels of the proper sizes were cut from the boards and some of the edges were bound with crimped sheet metal. They were used to replace the veneer in about 300 wire-bound containers of standard design. The filled containers were tested by the company's Package Research Laboratory, which found that their performance was equal and in some respects superior to that of similarly loaded containers made from wood veneer. Results of this work have been made available to the industries concerned.

The type of straw pulp and the method of forming boards from it are essentially the same as were used for making strong insulation board from straw, as previously announced by the Laboratory, and the basic research for insulation board is directly applicable to the development of the boxboard. No new types of manufacturing machinery are required for the boxboard process.

State agencies in Kansas, Nebraska, and Colorado have become interested in the potential use of wheat straw in paper and board manufacture, and each State has published facts on possible locations for mills where surplus straw is available in large quantities, together with labor, fuel, electric power, and water.

A prominent engineering firm estimated the cost of a plant to process 150 tons of straw per day, with a daily output of about 123 tons of board, at $3,876,000, and the total manufacturing costs at $54.40 per ton of board produced. The corresponding cost per 1,000 board feet would be $77. On the basis of this price, in comparison with the December 1950 price of $110 per thousand board feet of wood veneer, f. o. b., southern mills, it was estimated that the strawboard panels, including the metal edging required to prevent cutting by the binding wires, could be manufactured and sold in competition with veneer at a profit of 26 percent before deducting Federal income tax. Veneer was priced in February 1951 at $120 to $140 per thousand board feet.

A number of companies engaged in the manufacture of wire-bound shipping containers are interested in the possibility of building a mill

for the manufacture of straw boxboard. It would be practical in such a mill to combine the manufacture of boxboard and insulating board products from wheat straw.

Composition of New Commercial Corn Hybrids Determined

Industrial uses and the feed value of corn depend on its chemical composition, especially its content of starch, protein, and oil. Although protein and oil are present in much lower proportions than starch, their industrial value per pound of isolated component is considerably greater. In the development of new corn hybrids, plant breeders have placed major emphasis on the improvement of agronomic characteristics, such as yield and resistance to disease and lodging. Less attention has been given to chemical composition, particularly by private seed-corn companies. As the new varieties developed or introduced by these companies will constitute the commercial corns grown by farmers, it is advantageous to know their composition as early as possible in the developmental program. Such information will show the influence of current breeding programs on composition and may indicate limitations on corn yields imposed by either a higher protein or higher oil content.

Opportunity for such a study was provided the Northern Regional Laboratory by collaboration with the Illinois Agricultural Experiment Station in its annual corn performance test. Some 300 varieties of corn submitted by private seed-corn companies, as well as some hybrids developed by the Station, are grown each year on 8 fields at different locations in the State. For the past 3 years the Northern Laboratory has analyzed samples of corn from these plantings, and some significant conclusions have been drawn from the data on composition.

Varieties relatively high in protein or oil for any one year were also high in other years. The actual content of oil and protein, however, varied markedly with the season and with soil fertility. No effect of latitude was observed, perhaps because most of the varieties grown at any location were adapted to that latitude. There was no correlation between yield and protein or oil content. In any area high-yielding varieties may be selected or developed that are also relatively high in oil and protein. Three-year averages showed protein content of the varieties ranged from 8.6 to 11.7 percent and oil content from 4.3 to 4.8 percent. Several varieties had higher contents of both constituents than the average found for corn sold on the principal markets, indicating that some improvement in composition can be made by proper selection from varieties available.

Pollen Source Has Little Immediate Effect on Protein Content of Corn

Analytical studies of the composition of new hybrids developed by experiment stations and commercial breeders led the Northern Regional Laboratory to question the effect of cross-pollination among varieties grown on adjacent experimental plots. To determine the immediate effect of pollen source on the protein content of corn, a co-

operative experiment was carried out with the Illinois Agricultural Experiment Station. Low-protein and high-protein strains of corn were pollinated with a mixture of pollen from high-protein and low-protein strains which differed in the color of their kernels. Thus different pollen parents produced kernels of different color on the same ear, and the parentage was indicated by kernel color. Analyses of 200 pairs of samples showed that the pollen source had little effect on the protein content of the grain. Therefore, composition of corn grown in plots adjacent to other varieties is representative of the variety planted. Accordingly, protein analyses, such as those reported for the Illinois Agricultural Experiment Station's annual corn performance test, in which commercial hybrids are evaluated, can be accepted as representative of the varieties planted.

Effects of Maturity and Drying Temperature on Composition of Corn Determined

High-moisture corn may result from frost damage to the immature crop, unfavorable weather conditions during harvesting, or the common tendency to use mechanical harvesters before field drying is complete. In recent years, artificial drying at elevated temperatures has been used to reduce the moisture content of such corn to safe storage levels. Industrial consumers, however, have generally objected to the processing characteristics of artificially dried corn. Questions have arisen concerning the composition of corn as affected by maturity at harvesting and temperature of drying.

In a cooperative project of the Northern Regional Laboratory and the Illinois Agricultural Experiment Station, the effects of these factors on composition were determined. Corn was harvested 20 days after pollination and at intervals thereafter so that samples were obtained with moisture contents of 70, 54, 47, and 28 percent. Immediately after picking, portions of each sample were frozen to inhibit any changes in composition resulting from enzyme action. The composition of these portions, vacuum dried from the frozen state, provided controls for comparison with the remaining portions of each sample which were dried at room temperature and at 110°, 130°, and 180° F.

It was found that protein, sugar, ash, and the vitamins niacin, pantothenic acid, biotin, and riboflavin decreased as the corn matured, whereas starch, oil, and the vitamin pyridoxin increased with maturity. Sugar, which is present in relatively large amounts in immature corn containing more than 50 percent moisture, appears to be largely converted to starch during drying at room temperature, but to a lesser extent at elevated temperatures. Compared to drying at room temperature, artificial drying of immature corn at temperatures above 130° F. minimized loss of niacin, but increased the loss of pantothenic acid and pyridoxin. Most significant was the finding that the composition of corn harvested with less than 40 percent moisture was little affected by the temperature of drying.

New Chemicals Made From Corncobs and Other Residues via Furfural

Furfural is an oily liquid having an odor of bitter almonds. It is made by cooking plant materials that are rich in pentosans (polymers

of five-carbon sugars) with strong mineral acids. The best raw materials for furfural manufacture, from the standpoint of availability, composition, cost, and ease of processing, are corncobs, oat hulls, cottonseed hulls, and rice hulls. All of these crop residues are now used for this purpose.

Furfural has important industrial chemical uses, such as in the refining of lubricating oils, the fractionating of rosin and vegetable oils, and the purification of butadiene for synthetic-rubber production, and as a component or intermediate in the manufacture of synthetic resins. Its most recent and possibly most important use is as a raw material for the manufacture of a nylon intermediate. This last use is of particular interest because it represents the first large-scale utilization of furfural as a basic chemical intermediate that actually goes into and becomes part of a product for sale to the consuming public.

Research investigations concerned with expanded utilization of agricultural residues at the Northern Laboratory have included a program designed to discover and develop new and expanded uses for furfural as a chemical intermediate. From this work have come processes for the conversion of furfural into new as well as previously known chemicals from which vitamins, antimalarial drugs, essential amino acids, plastics, and plasticizers can be synthesized. Most recently a study of catalysts and process conditions has resulted in methods whereby the simple reaction products of furfural with acetaldehyde and acetone, both readily available, low-cost, and industrially important chemicals, can be converted by treatment with hydrogen and water into water-soluble trihydroxy compounds resembling glycerin in many of their chemical and physical properties. Treatment of these glycerinlike products with fatty acids yielded a series of new compounds—esters that resemble glycerides. The properties of these esters indicate that they may find uses as synthetic lubricants for aircraft instruments, as high-quality plasticizers for vinyl and cellulose acetate films, or as components of hydraulic fluids useful in automobiles and airplanes.

In addition, cyclic ether-substituted alcohols are obtained as coproducts with these trihydroxy compounds in the hydrogenation process as applied to furfural-condensation products. These, because of their unique arrangement of functional groupings, also appear to be promising intermediates for the synthesis of new solvents, plasticizers, and lubricants.

By variation and control of hydrogenation conditions it has been shown to be possible to convert the 3 simple condensation products of furfural with acetaldehyde and with acetone into 19 different chemical compounds. Preparation of derivatives and further processing reactions can easily result in the synthesis of hundreds of new chemicals basically derived from agricultural residues via furfural.

STARCH AND STARCH DERIVATIVES

New Transparent Wrapping Material Made Experimentally From Starch

Demand has steadily exceeded the supply of transparent films for wrapping foods, select articles of clothing, and other special items for the retail trade. Most timely, therefore, is the Northern Regional

Laboratory's development of a new transparent film from amylose, a constituent of the common starches.

Research under an RMA project has resulted in the development of a process for the laboratory preparation of high-quality amylose films. The process involves dissolving the amylose in hot water containing butyl alcohol, removing most of the alcohol by distillation, filtering while hot through a coarse fritted-glass disk, and spreading the hot water solution of amylose on polished glass or metal plates coated with a silicone preparation.

An outstanding characteristic of amylose films is their digestibility in the human body. This special property suggests uses in the food-packaging and pharmaceutical industries. Detailed investigation has been made of many of the other properties of the films. The dry tensile strength, flexibility, tear resistance, and bursting strength are comparable to those of commercial transparent wrapping materials. Heat treatment at high humidities improves the wet strength and flexibility of amylose films. The films are highly transparent to ultraviolet light but become somewhat brittle on prolonged exposure. Not only can the material be modified by addition of plasticizers, such as glycerol, but other modifiers or additives can be put in the film to adapt it to special uses. For example, the addition of certain dyes gives colored films that are decorative.

The relation between film properties and molecular size of the amylose indicated that severe breakdown of the polymer was detrimental to film properties. Likewise, incorporation of increasing proportions of amylopectin, the branched-chain starch component, led to a less rugged film. However, the dry tensile strength of film cast from a dissolved mixture of 76 percent amylopectin and 24 percent amylose (the proportions present in whole cornstarch) was high enough to suggest that useful films could be made from unfractionated starch if the mechanical strength requirements were not too stringent.

At present amylose is obtained in the laboratory by the fractionation of cereal or potato starches, which contain about one-fourth amylose by weight. Industrial applications of amylose films can be expected if economical means are found for obtaining amylose, either by improvement of the fractionation procedures, or by recovering starches of high amylose content from special plant materials other than those now used.

The excellent properties of amylose film, the simplicity of the process by which transparent film is produced from the amylose, and the relatively low cost of the starting material, starch, are factors that favor commercial production of the film. Coating of objects with amylose film can also be accomplished by spraying or dip-coating techniques.

New Acetates of Starch and Dextran Developed

The acetates of starch and its fractions, amylose and amylopectin, and the acetates of dextran, another versatile polymer of glucose that is produced when certain bacteria grow on cane or beet sugar, have not enjoyed wide industrial or research applications. The main reason was that the methods of acetylation used caused breakdown

of the products during their preparation. In addition, the acetates were not soluble in low-cost, commercially available solvents.

The lack of outlets for these acetates is in marked contrast to the wide market for cellulose acetate, which is used in large quantities in the manufacture of acetate rayon, nonflammable film, and plastics. As the result of recent experimental work at the Northern Regional Laboratory, however, the prospects are now more favorable for development of similar uses for the acetates of starch, amylose, and dextran.

Difficulties in the preparation of these acetates have been overcome by improvement of a previously known method of acetylation. The improved method is uniquely effective because of the use of a commercially obtainable organic solvent, formamide.

Starch, starch fractions, and dextran have been acetylated in a special dry state. In addition, satisfactory acetylated products have been obtained from dextran in the form of a wet, gummy mass (as isolated from the culture in which it was produced) and from the amylose paste that is isolated from starch solutions. In all cases acetylation was complete in about 18 hours at room temperature. When dry, the final products were white fibers or powders.

The acetates of the starch fractions, amylose and amylopectin, are soluble not only in chloroform and other halogenated hydrocarbons but also in acetone, a preferred solvent that costs less. The dextran acetates prepared by the new method were completely soluble in halogenated solvents. These practical and useful solubility properties have not been obtained heretofore. Also of much practical importance is the fact that the molecular size of the starch and dextran acetates was not reduced by the reagents used in the method of preparation.

Industrial Demand for Allyl Starch Increases

Previous reports have described progressive steps in the development and commercialization of allyl starch, an unsaturated ether derivative of starch that contains about 1.8 allyl groups per glucose unit and is useful as lacquer. Although starch itself is insoluble in almost every solvent, fresh allyl starch is readily soluble in organic solvents such as alcohol, acetone, benzene, toluene, and xylene. A solution of allyl starch can be brushed or sprayed on wood, glass, or metal, just like any other lacquer. After the solvent evaporates, a slow chemical reaction, involving oxygen of the air, takes place in the film and makes it insoluble in organic solvents and impervious to oils. The surface retains its original high gloss and becomes hard and resistant to temperatures up to 400° F.

Allyl starch has been used for furniture finishes, interior wood coatings, metal finishes, and heat-setting adhesives, and as a vehicle in printing ink. The printing-ink industry consumes a great deal of the allyl starch now produced.

Current laboratory work is concerned with the development of aqueous emulsions of allyl starch to avoid the cost and fire hazard involved in the use of organic solvents. Attempts are also being made to formulate allyl-starch pastes that can be diluted with water to form emulsions. Several emulsion recipes have already found commercial application. Some progress has been made in reducing the drying

time of allyl-starch films. For example, ultraviolet irradiation produces the same degree of hardness in 4 to 6 hours that is normally obtained in 24 hours.

The laboratory preparation of allyl starch was first described in this Bureau's report for 1944; and pilot-plant production of the material was announced by a commercial firm in 1947. This firm reports that the ever-increasing demand for allyl starch has required periodic increases in production facilities during the last 4 years. An important potential value of allyl starch is as an extender or replacement for shellac in the event that imports of shellac are interrupted.

Replacement Starch Provided for Nonfreezing Electric Dry Cells

In the manufacture of voltaic cells of the dry type found in some radio, telephone, and other communication equipment, in flashlights, and in weather-recording instruments, starch having certain properties is an important raw material. It is widely used in preparing a special coating that separates the outside zinc can (negative electrode) from the pasty mixture of carbon, manganese dioxide, and other chemicals that fills the can and surrounds the carbon (positive) electrode in the center.

When dry-cell batteries are manufactured for use at very low temperatures, a special process is necessary to prevent "freezing up" and failure to operate. In this process a starch that makes a paste of certain special properties must be used. High-quality sweetpotato starch made by a process developed by this Bureau had these properties. During World War II, and until recently in fact, this was the only product found satisfactory in the manufacture of dry cells of sufficiently high quality to meet the rigid requirements of the Signal Corps for use in equipment of the armed services in subzero weather.

The commercial production of sweetpotato starch ceased in 1947, because prices did not favor the use of this vegetable as an industrial raw material. The problem of a continuing source of satisfactory paste for electric dry cells for military use at very low temperatures became acute. The Southern Regional Laboratory was asked to study the situation and make suggestions based on its experience in the development of high-quality sweetpotato starch.

By applying available knowledge of the physical and chemical properties of sweetpotato starch, a formula was developed for a new product composed essentially of abundant domestic starches which was believed to have the properties needed for making nonfreezing dry cells. This mixture has now proved satisfactory for commercial production of high-quality dry cells that are acceptable to the Signal Corps for use in extremely cold weather.

Thus the Laboratory's research, which led to a successful method of producing sweetpotato starch under favorable economic conditions, has also provided a replacement for sweetpotato starch for use in an essential industrial and defense product when sweetpotato starch is not available commercially.

FERMENTATION PROCESSES AND PRODUCTS

Alcohol in Motor Fuel Does No Damage to Premium-Grade Lubricating Oils

In a study on the possibility of using alcohol as a motor fuel, conducted at the Northern Regional Laboratory, it has been recognized that alcohol, either injected into the manifold or used as a blend with gasoline, might have a detrimental effect on additives (antioxidants or corrosion inhibitors) in the premium grades of lubricating oil recommended for use in most passenger automobiles.

Work on this problem has been carried out by the Armour Research Foundation under an RMA research contract. In a series of tests, a standard Chevrolet engine was run in a manner to reproduce the effect of ordinary driving and of light delivery service on oil. Four lubricating oils being sold over a wide territory and containing different types of additives were used in the tests. Runs were made in duplicate, one with alcohol-water injection and the other without. The alcohol was injected as a mixture with water in equal parts by volume and at a rate to make the fuel consist of one part water and one part alcohol to eight parts gasoline by volume. That rate of alcohol consumption is higher than would be required under the load conditions of the tests.

Several methods of judging the performance of a lubricating oil are used, one of which is measurement of the weight lost by the test bearings during an engine run. Under the ordinary driving conditions, alcohol-water injection reduced loss in weight of the bearings. In two extreme cases the average loss with one oil was reduced from 476.5 to 101.5 milligrams and with another oil from 364.0 to 98.0 milligrams. Under simulated light-delivery-service conditions, alcohol-water injection slightly increased loss in weight of the bearings, presumably because less varnish was deposited on the bearings, but the average loss in weight of the bearings was below normal.

In these tests sludge deposits showed a tendency to be greater with the use of injection under simulated delivery-service conditions; however, no such tendency was shown under conditions simulating ordinary driving.

Within the limits of the investigation, it appears that injection does not interfere with the action of lubricating-oil additives but actually may improve their effectiveness in some respects.

Itaconic Acid Made by Fermentation

Flexible, strong, transparent plastics are needed for fabricating noses, domes, turrets, and windows of military aircraft and for optical equipment for military instruments. Although itaconic acid is not an important article of commerce at present, its properties are such that it could be used in large quantities in this type of plastic, if it were available at a reasonable cost. Excellent transparent plastics of the thermoplastic type can be made by the copolymerization of itaconic acid esters with methacrylates, vinyl chloride, or acrylonitrile. The resulting plastics have higher flexural and impact strengths than do those made without itaconic acid esters.

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Because of the potential uses for itaconic acid, the Northern Regional Laboratory has been interested for several years in producing it by fermentation of sugars with *Aspergillus terreus*. With mold strain NRRL 1960, it was found possible to obtain a yield of about 33 percent by weight in shake flasks. Difficulties in maintaining a high and uniform acidity showed the necessity of experiments on a larger scale. The experiments were therefore continued in 20-liter fermentors constructed of stainless steel. Besides the degree of acidity, other factors, such as the source and optimal amount of nitrogen for growing the organism, were reinvestigated. The yield was raised to about 55 percent, and the fermentation time was reduced.

Pilot-plant studies are now being conducted on the process. Yields of more than 60 percent, based on the anhydrous glucose used, are obtained in 72 hours or less in a simple medium containing corn sugar (dextrose) with small amounts of corn-steep liquor, magnesium sulfate, and ammonium sulfate. The initial pH of the medium is adjusted to 4.5 with itaconic acid. Optimum fermentation rate and acid yield are obtained when the fermentation is conducted at 95° F. with 10 percent inoculum, moderate agitation and aeration, and pressure of 10 to 15 pounds per square inch gage.

The crystalline acid is recovered from the fermented liquor by filtering, evaporating the filtrate, centrifuging, washing, and drying. Ninety percent or more of the acid contained in the fermented liquor is recovered as a light tan product of 97-percent purity. A white product exceeding 99 percent in purity is easily obtained from the tan-colored acid by carbon treatment and recrystallization. The slightly impure acid is suitable for preparing esters of monohydric alcohols that are redistilled before use, while the white product must be used for preparing nonvolatile esters.

Based on the early results, a preliminary cost estimate indicates that itaconic acid can be produced and recovered by this process for 27 cents per pound in a plant producing 3 million pounds annually. Investment costs for this plant are estimated at $800,000. At this production cost, itaconic acid could compete economically with various dicarboxylic acids, especially unsaturated acids such as maleic and fumaric which sell for about 37 cents per pound. Itaconic acid esters could compete economically with methacrylates, which vary in price from 37 to 60 cents per pound. They would most probably be compounded with methacrylates for the production of plastics.

Submerged-Fermentation Process Yields Citric Acid From Cheap Materials

Citric acid, amounting to about 35 million pounds per year, is now produced by fermentation of beet molasses, or a mixture of beet and cane molasses, in shallow pans. The desirability of a satisfactory deep-tank citric-acid fermentation is widely recognized. Moreover, to date no process has been reported whereby cheap raw materials can be utilized without expensive preliminary purification.

Several years ago the Northern Regional Laboratory discovered that the addition of methanol or ethanol, in a slightly toxic concentration, to a commercial glucose medium markedly improved the yield of citric acid in pan culture. Methanol, which is not readily assim-

ilated by the fermenting organism *Aspergillus niger*, is more satisfactory than ethanol, which is known to serve as a food source for the mold. The purification process, of course, entirely frees the citric acid from methanol.

The nature of the methanol effect is not entirely understood, but it has been demonstrated, in part under an RMA project at the Northern Laboratory, that the mold's tolerance to iron and manganese in the medium is greatly increased. In submerged culture without methanol, more than 1 milligram of iron and manganese per liter of medium greatly inhibited the fermentation. However, when 2 to 3.5 percent by volume of methanol was added to the medium, cornstarch, commercial glucose, and cane molasses, all of which contain iron and manganese, could be fermented to citric acid in submerged culture.

In submerged shaker-flask cultures the addition of methanol increased yields of the acid threefold to eightfold with commercial glucose, fourfold with finely ground corn, sevenfold with cornstarch, and twelvefold with blackstrap molasses. The yield of citric acid, based on the weight of sugar consumed, varied from 63 to 85 percent. From 100 grams of anhydrous glucose suppied, 70 grams of anhydrous citric acid was obtained in a 6-day fermentation in the shaker-flask cultures. Similar fermentations in stainless-steel vats were very promising, 60 grams of citric acid being obtained from 100 grams of glucose supplied. Yields from the lots of blackstrap molasses used were essentially the same as those from glucose. Optimum operation procedures have not yet been clearly established for large-scale fermentations.

Methanol at 35 cents per gallon would cost roughly 1 cent for each pound of sugar supplied. The cost of methanol required appears to be considerably less than purification of the sugar solution by ion-exchange treatment. Several commercial companies are interested in trying out the submerged-fermentation procedure for citric acid with addition of methanol.

Vitamin B_{12} Produced by New Fermentation Process

An initial report of research by the Bureau on the production of vitamin B_{12} through fermentation processes was presented last year. Further progress has been made at the Northern Regional Laboratory in efforts to obtain higher yields of the vitamin. Although vitamin B_{12} is recovered as a byproduct of the fermentative production of such antibiotics as aureomycin and streptomycin, there is still need for its large-scale production to supply the increased demands of the pharmaceutical and feed industries.

At the Northern Laboratory an entirely new fermentation process for the production of vitamin B_{12} with *Streptomyces olivaceus*, NRRL B–1125, has been developed under an RMA project. It replaces a less productive fermentation process developed with *Flavobacterium devorans*, NRRL–54. The particular strain of *S. olivaceus* employed was found among several hundred isolates of *Streptomyces* which were recently obtained from Japanese soil. Noteworthy also is the capacity of this organism to produce an antibiotic substance simultaneously with vitamin B_{12}. Although not yet identified, the antibiotic is believed also to enhance the nutritional value of feeds supple-

mented with the culture material. Culture media and conditions developed on a laboratory scale were found to give good yields in first trials with large-scale conventional equipment.

A feature of the process is the relatively simple and inexpensive medium composed of carbohydrate, mineral salts, and a source of nitrogen such as soybean meal, distillers' solubles, mycelium from the penicillin fermentation, peanut meal, or cottonseed meal. The fermentation is conducted in a deep-tank fermentor in which the medium is mildly agitated and aerated. Maximum yields of the vitamin are usually obtained in laboratory fermentors in 40 to 64 hours; however, industrial-scale fermentations are continued routinely for 3 to 4 days.

The vitamin B_{12} is recovered in dried preparations of the fermented culture medium. It is useful for the vitamin enrichment of livestock feeds. Feeding trials with poultry showed the preparation to supply the vitamin B_{12} needs, plus an additional growth stimulation believed to be due to the antibiotic substance.

Cultures of *S. olivaceus* and directions for producing vitamin B_{12} were released for general use in December 1950. Approximately 70 requests for this material have been received from research laboratories and commercial firms, both here and abroad. At least two domestic firms were immediately successful in the use of the Northern Laboratory's process for quantity production of vitamin B_{12} concentrate. Still other firms have made successful preliminary fermentations and are preparing equipment for large-scale production of the vitamin. An application is pending for a Government-owned patent to fully protect licensed use of the process.

SUGAR PLANTS, SUGARS, AND SACCHARINE PRODUCTS

More Learned About Organic Acids in Sugar Beets

Improvement in sugar-beet technology requires more complete knowledge of the composition of the beet than is now available. With adequate knowledge of the nature and amounts of substances that interfere with the crystallization of sugar, development of rational methods for removing the impurities should be possible. Preliminary work showed that nonnitrogenous organic acids accounted for a large proportion of the nonsugar soluble solids in beets. Their separation and analysis by classical chemical methods would have been time consuming, and in some cases, inaccurate; therefore, a different approach to the problem was required. This study has been undertaken as an RMA project at the Western Regional Laboratory.

Recent advances in chromatography offer a new way of separating small quantities of many compounds in pure form but not in adequate amounts for identification and accurate analyses. Two of the new chromatographic techniques, paper chromatography and resin-exchange reactions, have been applied to the problem of sugar-beet analysis with considerable success.

In the first step—paper chromatography—solutions containing an amount of acidic material too small to be weighable on the ordinary analytical balance are placed near one edge of a sheet of filter paper. An organic solvent capable of dissolving the acids is allowed to diffuse from that edge to the other end of the paper. The acids move different distances, depending on their relative solubility in the applied solvent.

After diffusion the paper is dried and the organic acids are located with acid-base indicators. The distances moved by unknown acids are compared with those for known acids. When they are the same for a given pair of acids with two or more organic solvents it may be assumed that the acids are identical.

The next step, which is accomplished with ion-exchange resins, is to isolate larger amounts of a tentatively identified acid. A portion of strong anion exchanger is adjusted to an acid concentration that is satisfactory for separation of weak acids, and another portion is adjusted for strong acids. The second portion is placed in the lower part of a column, and the first in the upper part of the same column. Sugar-beet juice pretreated to remove nitrogenous compounds is passed through the column. The adsorbed material is then slowly eluted with a dilute solution of strong acid. As liquid flows from the column, it is collected as a series of small samples. Each sample is tested by paper chromatography. All samples containing the tentatively identified acid are combined. Isolation of pure acid from the combined fractions is readily accomplished.

The combination of the two techniques has been so successful that nearly all the organic acids in sugar-beet juice have been separated in pure form, with only trace amounts of some acidic impurities remaining to be identified.

The major organic acids identified and analyzed in sugar-beet juice are lactic, citric, malic, and oxalic, with pyrrolidone carboxylic acid appearing in the processing liquors. Glycolic, glyceric, and succinic acids are present in small quantities. Through the cooperation of the beet-sugar industry, processing liquors were obtained from nine factories in the principal sugar-beet-growing areas. Examination of these liquors showed that only citric and oxalic acids are removed by currently used processes. Consideration is now being given to ways of removing more acids, so that the yield of sugar can be increased.

The chromatographic procedures developed for the organic acids of sugar beets may be useful in studies of composition of other agricultural commodities, fermentation liquors, and biological fluids.

New Method Developed for Using Two Sugarcane Byproducts

The cane-sugar industry needs new sources of income to maintain profitable operation in the face of increasing costs. Sugar-milling operations are so efficient that further process improvements offer little opportunity for significant cost cuts. However, improvement seems possible in the utilization of the industry's primary byproducts—blackstrap molasses and bagasse (cane refuse after the juice is extracted for sugar making). The Northern Regional Laboratory has proposed a method for such improvement.

Most mills utilize the byproduct bagasse in only one way—for fuel. However, advances in sugar-house heat economy leave a large surplus of bagasse that is not needed to generate steam. Usually this excess is burned simply to dispose of it. Several sugar mills in the United States bale bagasse and sell it to the insulating-board industry at a small profit. A few mills convert the byproduct into such commodities as plant mulch and chicken litter. In all, however, only a negligible proportion of the total bagasse in the world is used profitably.

Blackstrap molasses, the second cane-sugar byproduct, has not

found a stable market price for years. Although its use for livestock feeding is increasing, its price has been based on the alcohol market. The sugar industry wants to tie blackstrap molasses in with the feed market because its highest value is for feed.

The most satisfactory solution of byproduct problems is for the industry producing the byproducts to take upon itself the task of converting them into high-grade merchandise with reasonably stable and larger markets. All the successful industrial uses of bagasse and blackstrap have been developed independently of the sugar industry. Making the most of the combination of blackstrap and bagasse is obviously the task of the sugar mill. The results of cost studies and small pilot-plant work at the Northern Laboratory point to the joint conversion of blackstrap and bagasse into new, higher-grade products as the best solution of the sugar mill's byproducts problems.

Basis of the conversion process proposed by the Laboratory is the method it has developed for separating pith and fiber directly as the bagasse comes from the cane mill. Both pith and fiber are then dried. The quantity of pith is sufficient to absorb all the molasses, forming a nonsticky mixture that can be used as a component of animal feeds. The dried and baled pith-free fiber is available for sale to paper mills.

Although the method has been tested only in the Laboratory's pilot plant, the preliminary data have been presented promptly to the industry. Engineering design and data on operation are being obtained for large pilot-plant installations at sugar mills.

Estimates of costs and profits of the process are based on assumptions which must be further verified. Indications are, however, that the operation may give the sugar mill an additional profit of near $1 per ton of cane processed.

When the pith-free bagasse, obtained by the new method, is pulped it yields a superior material for making paper—an especially significant discovery in view of the serious pulp shortage developing in the United States. The pith-molasses mixture appears to be a usable feed for cattle, particularly at a time when cattle production is increasing in the South.

Processing of New Sugarcanes Studied

New high-yielding, disease-resistant varieties of sugarcane, and varieties with improved adaptability to mechanical harvesting, are being planted by more and more growers. This change to new varieties makes obsolete much of the existing information on the processing and milling characteristics of commercial sugarcanes. From earlier experience it can be assumed that existing information cannot be used as a guide to the behavior of the newer canes as to efficiency of juice extraction, effectiveness of clarification, and yield of sugar. This has been demonstrated repeatedly in commercial operations, but such operations are on too large a scale to determine the cause. Commercial mills necessarily handle mixtures of canes of widely different qualities, so it is not practical to study the juice from any particular lot separately.

For this reason the Bureau, under an RMA project, has made arrangements to conduct complete pilot-plant investigations in order to get detailed information on the effects of special properties of the new

canes and their juices upon the recovery of sugar. These arrangements include an RMA contract for milling tests by Louisiana State University at Baton Rouge, followed by studies of the subsequent processing steps by the Southern Regional Research Laboratory. These studies are conducted jointly in a special pilot plant provided by the University.

Experiments can be carried out on individual lots of cane weighing as little as 2 tons; yet the processing is continuous, and operating conditions are automatically controlled to simulate those of full-scale sugar manufacture. In this way it is possible to study the processing behavior of juice from a particular lot of cane, and to correlate results of the experiment with the composition of the cane and juice used. The relatively small amount of material required also makes it possible to conduct tests on some of the more promising unreleased varieties of cane to obtain advance information on any peculiarities of composition that could affect ease of processing and recovery of sugar.

In the program conducted in the 1950 grinding season, two or more experiments were made on each of eight varieties of cane supplied by the American Sugar Cane League. These varieties included the newer commercial varieties in Louisiana and two that have not yet been released for commercial planting. The behavior of these canes in milling, including the power required for grinding them and the efficiency of sugar extraction, were determined in the milling tests conducted by staff members of Louisiana State University.

Juice obtained from each variety was collected and kept at a low temperature to permit continued processing at a rate of about 50 gallons per hour. In the small-scale liming and clarification equipment, the experiments conducted by the Southern Laboratory revealed differences in rate of clarification, volume of impurities precipitated, and clarity of the juice. In some of the experiments observations were made on the ease of filtering the precipitate obtained in clarification, on the ease and completeness of crystallization of sugar, and on the quality of raw sugar produced. These data were correlated with analyses of the original juice and of the sirups and molasses resulting from its processing. The information was published promptly to help the operators of Louisiana sugar mills.

Thus far attention has been given primarily to a comparison of the newer varieties of cane. A broader objective of the investigations is the study of the exact manner in which milling and processing generally are affected by the composition of surgarcane and of the juice produced from it under various milling conditions. With increasing knowledge on the minor constituents of cane and juice and the development of new analytical techniques, it will be possible to determine precisely which constituents or classes of substances are responsible for difficulties in the clarification of juice and crystallization of sugar. This will serve as a basis for developing methods to overcome the difficulties and to obtain more effective clarification, greater recovery of sugar, and better quality.

Knowledge of Composition of Sugarcane and Products Expanded

While the mineral (ash) constituents of sugarcane, cane juice, molasses, and sugars have been extensively studied, knowledge of the organic nonsugar constituents has remained limited. Yet such knowledge is greatly needed as the basis for a better understanding of the

chemistry of sugarcane processing, which should lead to improvements in the operations involved. During the past year the Bureau began investigations under an RMA project to fill this gap in the available information by the application of modern research methods.

The results of the new approach to the problem already have more than doubled the number of simple organic nonsugar substances known to be present in sugarcane juice, and are expected before long to provide the data necessary for characterizing the more complex substances.

The studies have been made partly under an RMA research contract by Ohio State University and partly by the Southern Regional Laboratory.

Scientists at the university isolated several complex polymeric substances containing nitrogen from molasses made from domestic sugarcane juice in which tryptophan and lysine, the simpler amino acids, were known to be present. Using ion-exchange and chromatographic methods, they were able to detect at least small amounts of glutamic acid, glutamine, serine, glycine, alamine, and gamma-aminobutyric acid in this same juice. Isolation and complete identification of these hitherto undetermined constituents of cane juice are now in progress. New information of this kind on the nitrogen-containing amino acids is important, because these constituents, during processing, are believed to form complex substances that increase the amount of molasses and decrease the amount of sugar produced.

At the Southern Laboratory studies were directed toward the isolation and identification of the organic acid constituents of cane juice that do not contain nitrogen. This work was done in connection with studies of aconitic acid, which is the principal organic acid constituent of sugarcane and which, by means of a process developed by the Bureau, is now being recovered commercially as a byproduct of sugarcane processing. In addition to aconitic acid, only malic, oxalic, and possibly citric acid had been previously identified in the nonvolatile matter isolated from sugarcane juice. By chromatographic fractionation on a silica gel column the Laboratory has now effected the separation of not less than five other organic acids of this class. Two of them have been purified by crystallization and definitely identified as succinic and fumaric acids.

This investigation has disclosed certain errors in conclusions reached by the application of older and more cumbersome analytical methods. Scientists formerly thought most of the aconitic acid in molasses was lost in the course of fermentation for the production of alcohol, and that succinic acid found in the still residues was a product of the fermentation. Definite information to the contrary is now available. By means of its own accurate method of determining aconitic acid, and by the application of new chromatographic methods, the Southern Laboratory has shown that approximately 85 percent of the aconitic acid in molasses can be found in the still residues after fermentation to alcohol and that succinic and fumaric acids, originally in the molasses, also accumulate in the fermentation residues.

Improved Candies Made for Military Rations

This Bureau, in cooperation with the National Confectioners' Association, has worked closely with the Chicago Food Laboratories of the

Quartermaster Food and Container Institute on the development of improved candies for use in rations for the Armed Forces.

There is need for confections that combine high concentrations of various nutrient substances with greatly improved storage life and acceptability to the soldier in the field. A wide variety of candies, as nearly as possible like the candy bars and other confections to which the troops were accustomed as civilians, is wanted. The problem is to prepare such popularly acceptable candies to meet the packaging and storage requirements of the Quartermaster Corps. The candy must keep safely for 6 months at 100° F. or 2 years at or below 70° F. Much of the candy for the Armed Forces will be packed in cans, and therefore must be in disk form. Moreover, the candy should be of kinds suitable for starch molding in order to take advantage of the large capacity of the confectionery industry for producing molded candies.

Among the improved candies developed thus far at the Southern Regional Laboratory for evaluation by the Quartermaster Corps are disks of fudge, caramel, and caramel-nougat combination.

Sorbitol, a new ingredient that is obtained by the hydrogenation of corn sugar, has been added to the fudge to keep it from drying out, which is the principal cause of deterioration in this candy. A fudge of excellent texture, which should retain its smoothness much longer than standard fudge preparations, is produced by the use of 10 percent sorbitol, with proportional reduction in the amounts of sugar and corn sirup.

The new caramel, which is of such outstanding quality that the Quartermaster Corps considers it particularly desirable for military rations, resulted from experiments with the solids from sweet whey, recovered in dry powdered form as a byproduct of cheese manufacture. This candy is prepared in such a way that it can be cast in starch molds in the disk form required for packaging in cans. It can thus be produced rapidly and with little labor.

The combination candy in disk form, which is very similar in other respects to a high-quality candy bar, was made by using a layer of nougat with some of the caramel to which dried sweet whey was added.

The coating on all these candies is a regular commercial summer-type coating chocolate that withstands heating at 110° F. for 2 years and does not melt readily when the candy is eaten. Further research is directed toward the development of a chocolate coating of better eating quality that will also meet the Quartermaster Corps' severe temperature requirement.

Storage tests are in progress to determine whether the high initial quality and acceptability of the candies so far developed will be retained when they are subjected to the tests applied to ration items.

Progress Made in Investigations on Maple Products

Fundamental studies on the composition of maple sirup and maple sap, first reported in 1949, have been continued at the Eastern Regional Laboratory under an RMA project. This basic information is necessary to an understanding of the development of flavor and color in maple sirup. Sirup and sterile sap have been fractionated into acid, basic, and neutral fractions. Fourteen organic acids have been found

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in sap and 14 in maple sirup, but only 9 are common to both. Only a few of these acids have been identified thus far.

The high-flavored maple sirup described in the 1950 report is now being manufactured in limited amounts by at least four commercial firms and one farm cooperative.

A new maple-sirup thermometer of the bimetallic dial type has been developed with the cooperation of a thermometer manufacturer, and is commercially available. Because of its open, easily read scale, it is far superior to thermometers previously used and will be an aid to producers in processing sap to sirup.

Preliminary studies of the development of color in maple sirup indicate that the final color depends primarily on the time required in boiling maple sap to sirup and that the production of light-colored, high-grade sirup will be favored by rapid evaporation, skillful operation, and sap of high sugar content.

A complete maple-sap evaporator of the usual kind has been installed at the Eastern Laboratory for the purpose of studying the various economic and engineering factors involved in the production of maple sirup in farm-type equipment. The evaporator has been provided with an oil burner as a source of heat, instead of the more common wood fire, in order that the heat input to the evaporator may be more closely controlled.

By the operation of this apparatus during the coming year, on both sucrose solutions and maple sap now held in frozen storage, economic and engineering data of value to the industry in determining the cost of producing maple sirup under various conditions will be obtained.

A Maple Products Conference was organized and held at the Eastern Laboratory in November 1950. The conferees came from 13 States and Canada, representing all phases of the maple-sirup industry, including producers, processors, research workers, farm cooperatives, and State and Federal agencies. This was the first meeting of its kind in the history of the industry. Twenty-four papers were presented, and common problems discussed. The conference, in addition to stimulating cooperation and interest in maple problems, led to the organization of a maple-industry advisory committee and to an intensified extension-service program in States where previously little or no extension work had been conducted among the maple-sirup producers.

New Uses Sought for Honey

Research on food and nonfood uses of honey was continued at the Eastern Regional Research Laboratory under an RMA project. The purpose was to provide as many market outlets for honey as possible, so beekeepers will be encouraged to keep enough bees to insure adequate pollination of various crops.

In order that as much honey as possible may be withdrawn from hives, large quantities of sugar are fed to bees at certain times of the year. This is done even when the darker, more strongly flavored, and less marketable types of honey, which can be used for this purpose, are in surplus in some areas. The primary reason for this anomaly is that shipping such honey from one area to another may spread American foulbrood, a serious bee disease. In a study of methods for sterilizing this honey, attempts were made to combine the action of germicides

and mild heat. Sterilization was achieved, but, when the sterile honey was fed to bees, too many of them died. The attack on this problem was then shifted to the use of heat alone—high temperatures for short periods. Fundamental data on the heat resistance of the spores of *Bacillus larvae*, the cause of the disease, have now been obtained and are being used to develop an inexpensive continuous process and to design equipment for its application.

An investigation of the potential place of honey in commercial baking was undertaken under an RMA research contract by Kansas State College. A report covering the investigations on bread is in preparation. Other phases of the research include fruit cakes, yeast-raised sweet goods, and several types of commercial cakes, where the valuable moisture-retaining properties and the desirable flavoring possibilities of honey should be of great value. It has definitely been confirmed that richer flavor, better palatability, and superior moisture retention result when 40 percent of the sugar in a standard commercial yellow-cake formula is replaced by honey. When honey replaced other sweeteners in standard commercial white bread, it was detected in the bread by most members of a taste panel, even at the 3 percent level. Certain floral types of honey were shown to be more desirable than others for use in white bread.

A modification of the honey-fruit spread described in last year's report makes it possible to manufacture this product without the use of expensive vacuum concentrating equipment. Commercial fruit-juice concentrates can be mixed with honey of good flavor and crystallized to give a fine-grained honey-fruit spread in which both fruit and honey flavors are recognized.

One of the difficulties in marketing liquid honey is its tendency to granulate to a semisolid mass by the formation of crystals of dextrose, one of the sugars in honey. Controlled heating to melt all possible seed crystals of dextrose is the method used to check this tendency, but the absence of any practical method for controlling the heating has probably caused overheating, with consequent damage to the flavor of the honey. A device intended to facilitate routine examination of honey for the presence of the undesired crystals during processing and in storage has been developed. Based on the old principle of enhanced visibility of many crystalline materials by polarized light, the instrument permits ready detection of a single crystal in a jar of honey.

Glass Color Standards Developed for Extracted Honey

The Eastern Regional Laboratory, in collaboration with the Production and Marketing Administration, under an RMA project, has developed permanent glass color standards for the classification of extracted honey for color.

Color has long been an important factor in the marketing of extracted honey. For many years an instrument known as the Pfund wedge comparator was used for grading honey. This device is satisfactory as a laboratory instrument but is impractical for field use. Consequently there has been great need in the industry for a simple, inexpensive grading device. This need has been met by the new glass color standards and the simple comparators in which they are mounted.

The color standards are amber glasses that have been ground and polished to specified thicknesses, based on spectrophotometric measurements, so that their colors closely match the colors of typical clarified honeys which correspond to the grade boundaries of the Pfund instrument. The six glass color standards, representing the grade limits, are now the official United States Department of Agriculture color standards for extracted honey, and are designated Water White, Extra White, White, Extra Light Amber, Light Amber, and Amber. The standards are mounted as windows in metal boxlike comparators, so constructed that a bottle containing the honey to be graded is placed between two adjacent standards. A complete color-grading set consists of the following: Two comparators, each containing three color standards; square bottles with an inside diameter of 1.24 inch for honey samples; square bottles filled with distilled water for use as clear blanks behind the glass standards; and square bottles filled with water suspensions of bentonite to replace the clear blanks in grading cloudy or turbid honey. The color-grading sets are now commercially available and are in use by Government inspection services and by buyers and producers of honey.

ALFALFA PRODUCTS

Alfalfa Products Improved by Chemical Stabilizers

Continuing studies under an RMA project at the Western Regional Laboratory on the preservation of carotene (provitamin A) in alfalfa by means of antioxidants during drying and storage have shown that 2,5-ditertiary butyl hydroquinone and 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline are the most promising antioxidants out of about 300 tested for the purpose. Patent coverage has been granted on the use of the dihydroquinoline and its derivatives for the stabilization of carotene in alfalfa and other forage crops. Synthesis of a series of new compounds, in which the 6-ethoxy substituent of the basic 2,2,4-trimethyl-1,2-dihydroquinoline nucleus was replaced by 6-methoxy, 6-butoxy, 6-hydroxy, 6-methyl, 8-methoxy, 8-ethoxy, or 8-methyl, showed that some of the compounds were about equal to the 6-ethoxy derivative in carotene-stabilizing action but that none was superior.

Antioxidants are usually applied to alfalfa through the use of soybean, cottonseed, rice-bran, or other vegetable oil as a carrier. As the dihydroquinoline compounds are highly soluble in vegetable oils, they can be applied in effective concentrations with the minimum amount of oil required for dust abatement, which is about 8 pounds of oil per ton of alfalfa (0.4 percent). The 2,5-ditertiary butyl hydroquinone and N,N′-diphenyl-*p*-phenylene diamine, the latter compound presently used commercially for the stabilization of carotene in alfalfa, are much less oil-soluble and require about 20 pounds of vegetable oil per ton of alfalfa (1.0 percent) to dissolve enough antioxidant to give a final concentration of 0.015 percent of antioxidant when applied to the meal.

The application of the more effective antioxidants to dehydrated alfalfa is being studied with the Poultry Producers of Central California, a cooperative organization, at its plant in Ryer Island, Calif. The compounds are applied in suitable carriers to dehydrated alfalfa

under full-scale industrial conditions. Preliminary results indicated that the application can be made with relatively simple equipment. When alfalfa was treated with 0.015 percent of the dihydroquinoline compound in 1 percent cottonseed oil and stored for 6 months at room temperature the treated material retained 58 percent of the original carotene, while the untreated material was reduced to 31 percent. Alfalfa meal treated with the hydroquinone derivative retained 45 percent of its carotene under like conditions. Through the use of an oil as carrier, the extreme dustiness of the meal is reduced to a minimum and the desirable green color of the alfalfa is enhanced.

Before antioxidants can be used in a feedstuff they must be shown to be safe from the standpoint of toxicity, both acute and chronic. Toxicity tests on 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline and 2,5-ditertiary butyl hydroquinone are in progress in the Pharmacology Laboratory of this Bureau with rats as the test animal. Similar studies with chicks are being conducted cooperatively by the Colorado Agricultural Experiment Station, the American Dehydrators Association, and the Western Regional Research Laboratory. Under this agreement, funds furnished to the Colorado station by the American Dehydrators Association defray the major expense of the undertaking, while the Western Laboratory supplies the test materials and makes final chemical evaluation of the animal tissues. Tests have shown the acute toxicity of both compounds on rats to be low, and no significant symptoms of poisoning have been observed in rats fed the hydroquinone at 0.2 percent level over a period of 1 year. This is 20 to 60 times more antioxidant than would normally be present in prepared feed. Similar encouraging results are being obtained with the dihydroquinoline.

CHEMICAL PRODUCTS FROM TOBACCO

Activators Sought to Make Nicotine More Deadly to Insects

In its research on the chemistry and biological activity of nicotine, the Eastern Regional Research Laboratory has continued to search for synergists for nicotine as an insecticide. In the chemical sense, a synergist (sometimes called an activator) is a substance that, when mixed with a reagent, causes the mixture to have greater activity than the sum of the activities of the two used separately. Nicotine is an excellent insecticide, functioning in three ways—as a stomach poison, as a contact poison, and as a poisonous fumigant. Its present high price, however, restricts the agricultural use of nicotine, especially in competition with some of the newer synthetic insecticides. Low-priced synergists for nicotine, if useful in favorable ratios, would help bring nicotine back as an economic possibility. There is another economic factor to be considered. Nicotine is made from waste and scrap tobacco, principally midribs, or stems. With increased tobacco consumption there would be an increase in the supply of waste and scrap. But large inventories of nicotine would lessen the use for stems, thus creating a disposal problem for the tobacco processor. Cheap synergists might help to solve this problem by reducing the cost of using nicotine. Synergists could also play an important part in the Nation's economy in times of emergency, since the production of synthetic insecticides might be hampered by short-

ages of raw materials (sulfur, phosphorus, chlorine, benzene, etc.). Increased demands for nicotine would naturally result, and the use of activators would extend the available supply.

In cooperation with the Bureau of Entomology and Plant Quarantine and the Connecticut Agricultural Experiment Station, a screening program was developed to facilitate the search for chemicals to act as synergists for nicotine. Out of nearly 700 compounds tested, about 40 were selected for closer scrutiny. The entomological data on these compounds were subjected to a newly developed statistical analysis. Since the last report, in 1947, about 12 types of compounds survived the final screening. Among these is a whole series of commercially available compounds—monoesters of polyoxyethylene glycols, such as polyethylene glycol 400 mono-oleate. Monoethers of the type represented by tetraoxyethylene glycol mono-octyl ether also show synergistic activity. Another group of activators consists primarily of haloaromatic sulfides and aromatic dinitriles, such as bis-(*p*-chlorophenyl) sulfide and phthalonitrile, respectively.

With the synergists found thus far, the best ratio of activator to nicotine is about 4 to 1. All the toxicity data obtained have been the result of laboratory tests, and in some cases the tests were made on only one kind of insect. Field testing is now in progress.

VEGETABLES AND VEGETABLE PRODUCTS

Potatoes Dried by Natural Freezing

Natural freeze drying of potatoes apparently was originated by the ancient Incas of Peru. These Indians spread potatoes on the ground of the high plateaus, where they froze during the night and thawed during the day. Much of the juice was lost during the thaws.

The practice of the Incas seems to have been forgotten, but a group of potato growers in North Dakota, led by William M. Case, tried the idea in 1947. Potatoes mummified by alternate freezing and thawing in the field provided good feed for dairy and beef cattle. There remained a need, however, for data on the loss of nutrients resulting from the process and for demonstration trials in other potato areas, particularly Maine.

Under an RMA project, the Eastern Regional Laboratory cooperated with the Maine Agricultural Experiment Station in trials at Presque Isle in 1950. On February 1 potatoes were spread on the ground and also placed in open structures above the ground in layers of different thickness. By April 15 these potatoes had shrunk to half their original volume. By June 15 they had contracted to about one-third their original volume, were free from decay, and had an odor like that of silage.

Potatoes on the open racks lost 30 to 40 percent of their total solids between February 1 and June 15. Most of this loss was in substances other than starch. The nitrogen and mineral constituents of the potato juice that entered the soil greatly stimulated the growth of grass.

In laboratory experiments the greatest loss of juice (22 percent of the original weight of the potatoes) occurred during the first thaw. The loss on thawing decreased in subsequent cycles until only 1 percent was lost during the eighth cycle.

Microscopic examination showed that freezing of potatoes causes groups of cells to separate from other groups. After thawing, juice passes through the cell membranes that have been made more permeable and escapes through a network of canals between the groups of cells. Very little starch is lost in the process because not many of the potato cells are ruptured.

The indications are that potatoes freeze-dried in the field can be used for fermentations as well as for feed. The Northern Regional Laboratory obtained a good yield of alcohol upon fermenting potatoes that had been dried in this manner in Maine.

A second trial of freeze-drying in the field was made in cooperation with the Maine Experiment Station in the winter of 1950–51. Potatoes were spread on the ground in the fall and allowed to undergo alternate freezing and thawing until the following summer. Feeding tests will be made with the dried product. The results of these tests, with those of similar tests being made in Minnesota, will better enable the potato industry to judge the feed value of potatoes freeze-dried in the field. The freeze-dried product from 2,000 bushels of potatoes is being fed at the Northwest School and Experiment Station of the University of Minnesota at Crookston. Staff members of the Eastern Laboratory and of the Northwest School have maintained contact for prompt exchange of new information.

Rapid Volumetric Method Measures Sulfite in Dehydrated Potatoes

The present National Military Establishment specifications for dehydrated white potatoes require the presence of from 200 to 500 parts per million of sulfur dioxide to preserve their quality for food under conditions of high-temperature storage. The sulfite is added, after final preparation of the potatoes for dehydration, by spraying with or dipping in a solution of sodium sulfite or a mixture of normal sulfite and bisulfite. Where combustion gases from burning fuel oil are used directly for drying, the potatoes take up sulfur dioxide during the dehydration process. As the specifications provide for the sulfur dioxide content of the potatoes to be within a rather restricted range, it is necessary to analyze the product at frequent intervals for proper control of the sulfiting process.

The distillation method described in Quartermaster specifications is time consuming and not well adapted to processing-plant work where a minimum of equipment is desired. A rapid method for determining sulfite in dehydrated white potatoes has been developed and is now coming into use in place of the distillation method. The rapid method compares very favorably, from the standpoint of precision and accuracy, with the distillation method now in use, and both the elapsed time and working time have been cut, permitting an operator to make about four times as many analyses per day. The technique is simple and therefore suitable for use by nontechnical operators in dehydration plants and for testing for compliance with specifications. Standard laboratory equipment and common reagents easily obtainable are used. The usual method of sample preparation now in use is satisfactory.

For analysis, a representative portion of dehydrated potato is ground, and two samples are weighed into beakers. The samples are treated with a solution of sodium hydroxide. The mixture is stirred

gently, care being used not to beat air into the solution, and allowed to stand for 20 minutes. One of the samples is acidified with hydrochloric acid; starch solution is added; and the mixture is titrated immediately with a standard iodine solution to a definite dark-blue color. The titration is best made with a mechanical stirring device, including a propeller with broad blades that have a pitch steep enough to distribute the iodine throughout the solution without beating air into it. The volume of iodine used measures the sulfite content, together with any nonsulfite reducing materials. To determine reducing material other than sulfite, the second sample is acidified, and formaldehyde solution is added to bind the sulfite. While the mechanical stirrer is used for mixing, the solution is rapidly titrated, preferably with the burette stopcock fully open until near the end point and then with small increments added in quick succession until a dark-blue color persists for at least 15 seconds. The difference between the two titrations is a measure of the sulfite content of the dehydrated white potato.

Medicinal Chlorophyll Compound Prepared From Broccoli Leaves

Leaf meal prepared by dehydration of the waste from trimmed broccoli at Seabrook Farms, Bridgeton, N. J., under an RMA contract, has been successfully used as a source of medicinal-grade chlorophyll.

A chemical company in Pennsylvania has extracted 28 tons of this leaf meal and prepared sodium copper chlorophyllin from the extract by a method very similar to that developed at the Eastern Regional Laboratory and described in a mimeographed publication (AIC–299). This company normally makes chlorophyll salts from alfalfa leaf meal, but, because of the higher content of chlorophyll in the broccoli leaf meal (3 to 4 times as high as alfalfa), they have obtained much greater yields of the copper chlorophyll salt per ton of meal. This gives them greatly increased plant capacity.

Byproducts of the process are carotene and xanthophyll oils and a 38- to 44-percent protein meal, which has been found satisfactory as a feed supplement in broiler rations at the Delaware Agricultural Experiment Station.

Vegetable leaf meals high in chlorophyll can be utilized to better economic advantage for this purpose than directly in feeds. When used in this manner they still retain their high protein feed value.

Sex Hormones Can Come From Tomato Plants

Crystalline tomatine, a new glycosidal alkaloid isolated from tomato-leaf extracts in the Biologically Active Compounds Division, has been chemically degraded by acid hydrolysis to an aglycone portion, tomatidine, and a carbohydrate fraction consisting of one molecule each of xylose and galactose and two of glucose. Tomatidine has now been identified as a new steroidal secondary amine.

Part of the current interest in tomatidine lies in the discovery that it can be used as a starting material in the manufacture of the sex hormones progesterone and testosterone and possibly other biologically active sterols. An investigation initiated last year in cooperation with the National Institutes of Health led to this discovery.

Only three chemical steps are required to convert tomatidine into a sterol (pregnene derivative) that can be easily transformed into progesterone and testosterone.

Progesterone (used for correction of menstrual disturbances, prevention of spontaneous abortions, and relief in cervical cancer) and testosterone (also used as a remedy for menstrual disturbances, and for suppressing eunuchoidism and breast cancer) are widely known steroid sex hormones. The value of the new discovery lies in the fact that heretofore only three sterol sources were available for the preparation of progesterone and testosterone. These were cholesterol (isolated from animal-nerve tissue), stigmasterol (obtained from soybeans), and diosgenin (extracted from Mexican yams).

Syntheses from the first two sterols demand more chemical steps than the conversion of tomatidine. In the case of diosgenin, synthesis is relatively simple, but the Mexican yam does not grow in this country. Thus tomatidine, derived from the leaves, stems, and roots of tomato plants, promises to be a domestically available source of sterol for producing the steroid hormones by an inexpensive method of synthesis. This sterol might even be converted to cortisone.

To produce cortisone, however, one crucial reaction is necessary—the introduction of an oxygen group in a particular position in the sterol structure. When a practicable method for introducing the oxygen group has been perfected, the use of tomatidine as a source of suitable sterol may make possible a considerable reduction in the price of cortisone.

Degradation products of tomatidine, in addition to the particular sterol referred to, have been prepared and are being tested for biological activity. Moreover, the complete chemical structure of tomatidine is being determined.

The Bureau of Plant Industry, Soils, and Agricultural Engineering is cooperating in this work by growing tomato plants in experimental plots for the production of tomatidine in order to obtain yield and cost data. The feasibility of producing tomatidine from tomato plants after the fruit has been harvested is being investigated.

Problems in Vegetable Dehydration Receive Attention

From the standpoint of agricultural research one of the most important preparedness problems concerns the science and technology of vegetable dehydration. The Bureau has greatly intensified and expanded its research on this problem.

Dehydrated fruits, eggs, dairy products, meat, vegetables, and numerous special dehydrated ration items constitute a large and essential part of the food supply required to maintain an army overseas. The shrinkage in volume of these foods upon removal of their water content effects important economies in packaging materials and in scarce shipping and storage space. The simultaneous reduction in weight is equally important. When these advantages can be accompanied by maintenance of palatability during long storage under adverse conditions and by convenience of preparation and use by personnel in the field, military procurement on a large scale is assured.

In World War II many of the Bureau's contributions to dehydration science and technology were applied on a large scale in the plants of

the dehydration industry. After hostilities ended most of these plants, particularly those concerned with vegetable dehydration, were closed. By common report, the military experience with most of the dehydrated products had not been entirely satisfactory, especially in palatability and convenience of use. The Bureau has maintained a few long-range projects aimed at basic understanding of the difficulties.

The rearmament program and the outbreak of hostilities in Korea imposed a new timetable for the solution of these outstanding problems, especially in vegetable dehydration. The Bureau established close liaison with the Quartermaster Corps and began an investigation of several matters of immediate concern. A comprehensive survey of published information on a relatively new product, potato granules or dehydrated mashed potato, was issued in mimeographed form. The storage stability of the commercial product was investigated, and laboratory studies of the characteristic processing steps were undertaken with the purpose of discovering the causes of certain troublesome quality defects. Other experimental work undertaken at the suggestion of the Quartermaster Corps included studies of the techniques of sulfiting dehydrated vegetables, the development of acceptable dehydrated green peas and string beans, the solution of several problems in the dehydration of sweetpotatoes, and the development of better analytical methods for production control and inspection.

During World War II the Bureau sponsored the publication of a Department bulletin summarizing dehydration technology for the benefit of plant operators. This bulletin, now out of print, is being completely revised; two of the rewritten chapters, on the principles of drying and the characteristics of tunnel dehydrators, have been issued as mimeographed separates. At the request of the Quartermaster Corps. and financed by a grant of funds from the Corps. a "preparedness study" of dehydration is being prepared in the form of a handbook that can be used by the Corps to inform industrial management if it becomes necessary again to expand the dehydration industry rapidly.

Dehydrated Vegetables Made to Keep Better

Current defense preparations have greatly increased the importance of dehydrated vegetables. Civilian markets for certain products, notably potato granules (mashed potato powder), also have been expanded. However, several deteriorative processes occurring during storage limit the usefulness of dehydrated vegetables. These changes include the development of off-flavors and off-odors, color degradation, impairment of rehydration characteristics, and destruction of vitamins. Comprehensive studies at the Western Regional Laboratory have provided quantitative information on a number of factors affecting the storage stability of dehydrated white potato, sweetpotato, cabbage, carrot, beet, and onion.

The most important type of deterioration is associated with the so-called browning reaction and was particularly troublesome to the Armed Forces in World War II. This type of browning is a reaction between protein or other nitrogenous constituents and certain sugars and, unlike enzymatic discoloration, is not prevented by blanching. Because it proceeds much more rapidly at elevated temperatures,

dehydrated vegetables may deteriorate very quickly under unfavorable conditions of storage in tropical areas. The reaction results not only in development of a brown color, but in the appearance of an unpleasant scorched flavor and resistance to rehydration.

Quantitative measurements showed that substantial protection against browning is obtained by treatment with sulfite solutions and by drying to a low moisture level. Protection adequate even for military purposes appears obtainable by combining these two treatments. Thus in one typical lot of dried nonsulfited white potatoes, detectable browning developed in 9 days at 100° F. Comparable sulfited samples at 4 percent lower moisture content withstood storage at that temperature for 160 days before detectable browning occurred.

Low moisture was found to be especially beneficial for dehydrated cabbage and onion. For these commodities, which are particularly susceptible to browning, the storage life at 100° F. was increased eightfold to tenfold by in-package desiccation—packing a drying agent in the final container with the dehydrated vegetable as described in the report for 1945. Very low moisture levels can be conveniently obtained by this process.

The rate of browning was found to double with an increase in temperature of only 6° or 7° F. Browning is thus remarkably sensitive to temperature, as the rates of most common chemical reactions double with a 12° to 18° rise in temperature. It was also established that in the low-moisture range the rate is approximately doubled by an increase in moisture content of 2 percent.

Packing dehydrated vegetables in a vacuum or an inert gas such as nitrogen or carbon dioxide was shown to retard destruction of ascorbic acid (vitamin C) and carotene (provitamin A) during storage. It also greatly retards the development of rancid odors and flavors, but has little, if any, effect on browning. Loss of vitamin C was also markedly retarded by lowering the moisture content.

These studies have demonstrated that dehydrated vegetables of greatly improved storage stability can be prepared by a combination of sulfiting, drying to low moisture content by in-package desiccation or otherwise, and packing in inert gas. Such high-stability products would be of special value for military use in areas where high temperatures prevail in storage dumps and warehouses.

Method Developed for Freezing Sweet Corn

Various corn-processing procedures were studied to determine the most economical method of producing a frozen product of high quality. In particular, a careful comparison was made between the product obtained when corn was blanched (scalded) on the cob prior to cutting and that obtained when the kernels were first cut off and then blanched. That the former method was preferable appeared likely because of the obvious possibility that losses of kernel constituents, for example, sugars and volatile flavoring components, would be greater from the cut raw kernels than from the blanched-on-the-cob kernels in which the starch and protein had been set to a gel before cutting. Because these materials, particularly sugars, are important in determining the quality of frozen sweet corn, any measures that would tend to minimize their loss would improve the quality of the

frozen product. On the other hand, the practice of cutting off the kernels and then blanching offers economies, as it is not necessary to provide space for the cob in the blanching equipment.

Experimental data to substantiate these ideas on the advantages of blanching on the cob over blanching of the cut kernels were meager. Accordingly, investigations were made to obtain data that would place a comparison of the two processing methods on a sound scientific basis. The work was carried out by the Western Regional Laboratory in cooperation with the Utah State Agricultural Experiment Station and the Irrigation Experiment Station of the State College of Washington. The Golden Cross Bantam variety was used in the experiments. Harvests were made at four levels of maturity with moisture contents of raw material ranging from 63 to 77 percent. At each harvest, samples were prepared by six processing procedures.

Chemical analyses and tasting showed highly significant differences between the frozen samples prepared by the two methods, the advantage being in favor of material that had been scalded prior to the cutting operation. Samples processed in this manner, although flumed after cutting, were higher in total solids, total sugars, and weight per kernel than were samples that had been cut and flumed prior to blanching and then flumed again after blanching (a process used in many commercial operations). The losses from original values were: Total solids, 9 and 33 percent; total sugars, 24 and 89 percent; and weight per kernel, 8 and 26 percent. The cob-blanching procedure, therefore, alters the kernel contents, probably by gelation of the starches and proteins, in such a manner that loss of soluble materials, which occurs when the raw kernels are cut prior to blanching, is minimized. As a result, loss of nutrient material is minimized and the over-all yield of frozen product from a given lot of raw material is significantly increased (indicated by increase in the average weight per kernel).

A panel of experienced tasters judged the cob-blanched samples to be significantly better in flavor than the kernel-blanched samples.

Thus the results of chemical analyses and tasting have demonstrated a distinct advantage of blanching corn on the cob prior to cutting. By this procedure, which is easily adaptable to commercial operation, a frozen product of higher quality is obtained in greater yield.

Tests for Maturity of Sweet Corn Evaluated

The quality of canned and frozen sweet corn is determined to a large extent by the maturity of the raw corn. Ability to harvest the crop at optimum maturity and to separate corn of different maturities in a lot of harvested corn is essential to efficient production and high quality. Processors therefore need an objective measure of maturity that is sufficiently accurate, rapid, and simple to permit its use in production and quality control. Starch and moisture content have been established as accurate indexes of the maturity of sweet corn, but usual methods for their determination are not sufficiently rapid to be useful in plant operations, and the fieldman's thumbnail test is still widely used to determine picking dates.

The relative merits of several objective methods for the determination of the maturity of sweet corn for processing have been evaluated by the Fruit and Vegetable Products Laboratory in Prosser, Wash.

On the basis of a three-season study, it was determined that measurement of the refractive index of juice expressed from a representative sample met the commercial processor's requirements of simplicity, reliability, and speed in the objective measurement of maturity.

A study was conducted in cooperation with the University of Idaho at Moscow to determine factors that affect the distribution of maturity within a single field lot of harvested sweet corn. The study showed a considerable variation in the relative proportions of young, prime, and mature corn in a single harvest. This variation is related to both season and field-run moisture content. There is also a considerable increase in total yield of cut corn as it becomes more mature. By considering the probable total yield and the proportions of each maturity grade expected at the different moisture levels, processors will be able to more closely regulate harvest schedules to obtain the greatest possible yield of optimum-maturity corn.

Pink Centers in Frozen Brussels Sprouts Explained and Prevented

The occasional appearance of pink centers in frozen brussels sprouts has caused concern in the frozen-food industry, as the presence of an abnormal color causes a lowering of the grade of the frozen product and, as a result, a lowering in the value. A search of the literature failed to reveal any information on the factors involved in the development of the discoloration. Hence a study of the processing variables was undertaken at the Western Regional Laboratory to work out a practical method of prevention.

The effect of degree of blanch on the quality of the frozen material received first consideration, from the standpoint of both over-all flavor changes and development of pink centers. For this purpose samples prepared from the same lot of raw material were blanched in steam for 2, 3, 4, 5, 6, 8, and 10 minutes. These samples, after evaporation-fog cooling, were packaged, frozen, and stored at −10° F. for 10 months. Enzyme assays were carried out on the samples at the time of processing and at the end of the storage period by methods previously developed in the Laboratory. Peroxidase was determined by a semiquantitative test developed for use in processing plants and also by a quantitative test, guaiacol and hydrogen peroxide being used as substrates in both tests. Catalase was determined by a quantitative test in which rate of disappearance of hydrogen peroxide was used as a measure of catalase activity.

At the end of the storage period, taste-panel evaluations were carried out on the cooked samples to determine natural flavor, off-flavor, and color of the centers. The results showed that all samples that had been blanched to a point sufficient to inactivate peroxidase maintained quality over the 10-month storage period at –10° F. On the other hand, samples that had appreciable amounts of residual peroxidase had a marked off-flavor, a decrease in natural flavor, and a pink coloration at the centers. These data indicated clearly that lowering of quality and the development of pink centers in brussels sprouts could be prevented by blanching the material to a degree sufficient to inactivate the peroxidase as determined by the assay methods used. Hence underblanching was responsible for the phenomenon in question, and the peroxidase test, as applied in these experiments, could be used satisfactorily as a test for adequacy of blanch of frozen brussels

sprouts. On the other hand, catalase, because of its greater heat lability and because of its apparent inactivation during freezing storage, was unsatisfactory for the purpose.

Subsequent investigation has shown that pink centers develop immediately after blanching. In the underblanched material the rate and degree of discoloration can be greatly enhanced, for laboratory purposes, by the addition of a dilute solution of hydrogen peroxide to the surface of the cut halves.

Blanching to complete peroxidase inactivation was found unnecessary for prevention of pink-center development, as samples that had only about 10 percent residual peroxidase retained flavor quality and failed to develop abnormal coloration at the centers after 12 months' storage at –10° F. Nevertheless, blanching to complete inactivation of peroxidase is advocated in order to insure a certain margin of safety in the operation.

Sweet-Pickle Formulas Standardized to Prevent Losses

A chart that gives cucumber-pickle manufacturers the exact proportions of sugar and vinegar required to put up sweet pickles so they will keep safely was worked out in 1950 by the Food Fermentation Laboratory in Raleigh, N. C., in cooperation with the North Carolina Agricultural Experiment Station.

Information of this kind has been greatly needed. The procedures formerly used were based, for the most part, on each packer's experience. They were far from uniform or reliable, as evidenced by frequent cases of gaseous spoilage—the result of too little sugar or vinegar, or both.

One of the limitations in establishing uniformly safe preservation practices has been the lack of a suitable test organism having a wide range of tolerance to sugar, acid, and combinations of these ingredients. The Food Fermentation Laboratory obtained a number of cultures of a yeast (*Zygosaccharomyces* species) from typical cases of sweet-pickle spoilage in the industry. When this yeast exhibited rather remarkable resistance to sugar solutions and vinegar, individually in various concentrations, and to mixtures of the two, the Laboratory subjected it to a thorough investigation under carefully controlled conditions. This investigation led to the development of what is believed to be the first preservation-prediction chart available to the pickle industry.

The chart has been tested in a commercial plant in the making of about 40 experimental batches of sweet pickles over a 2-year period. Pickles made with the proportions of vinegar and sugar recommended on the chart did not ferment; those made with less of the preservatives underwent gaseous fermentation and spoilage. Laboratory examination of each spoiled lot showed yeast to be the causative agent. No doubt this chart, now being prepared for publication, will find widespread use in the pickle industry for standardizing sweet-pickle formulas to reduce spoilage and save sugar.

Cucumber Softening by Enzymes Controlled in Pickle Plant

Research reported in 1950 suggested that the cucumber itself may play an important part in the undesirable softening that sometimes

occurs in this vegetable during the period of storage as salt stock which precedes the manufacture of brined pickles. This theory was based on fundamental studies in which the nature and occurrence of a pectin-splitting enzyme in the cucumbers themselves and in various parts of the plant were thoroughly investigated by the Food Fermentation Laboratory in Raleigh, N. C., in cooperation with the North Carolina Agricultural Experiment Station.

The fundamental data have now been confirmed in a commercial-scale experiment conducted in cooperation with a pickle manufacturer. Some 15,000 bushels of Palmetto variety cucumbers (produce or slicer type) from the fall crop of 1950 were brined during cold weather under conditions that precluded fermentation. When these cucumbers showed signs of softening within a few days after the stock was covered with a 10-percent salt brine, immediate tests were started to discover the reason.

The Food Fermentation Laboratory obtained samples from 15 vats of the brined cucumbers and analyzed them carefully along with samples of fresh Palmetto cucumbers obtained from two growing areas (Florida and South Carolina). A thorough study of all these samples, and a comparison of the results, demonstrated that the softening was caused by a pectin-splitting enzyme that occurs naturally in the cucumber. This particular enzyme was found in samples of brine taken from vats in which the softening cucumbers were stored and also in various sections of the brined cucumbers. In addition, it was found in different parts of the fresh, unbrined cucumbers of the same variety.

The immediate result of the study, of course, was the saving of the 15,000 bushels of cucumbers affected, but the findings are even more significant from a long-range viewpoint. After identifying the responsible enzyme, the Food Fermentation Laboratory found that it was sensitive to acid and could be destroyed by treatment with either lactic or acetic acid, or inactivated by heating the brined material. Prompt application of these treatments by the manager of the cooperating plant checked the softening before serious losses had occurred. This verification on a commercial scale of the data previously developed in fundamental research on the role of enzymes in the spoilage of cucumbers is another step forward in efforts to improve the processing of food products. The control methods applied in this particular study will be valuable to the entire pickle industry as situations involving losses from the softening of salt-stock cucumbers arise.

FRUITS AND FRUIT PRODUCTS

Basic Information Sought for Improving Citrus Fruit Juices

The Citrus Products Station at Winter Haven, Fla., has sought, under an RMA project, to acquire new basic information on problems involved in the pasteurization of canned citrus juices.

While much remains to be done, one phase of the work has progressed to the point of practical application. Cans filled with citrus juices may swell because the juice was inadequately pasteurized and ferments, or for other reasons. Serious losses may result unless the exact cause of the trouble is found and promptly removed. This can now be accomplished by applying a test developed for determining the effectiveness of the pasteurization treatment. When the juice is known

to be properly pasteurized, the search for the trouble is considerably simplified. The turbidity to which the citrus juice owes its characteristic flavor and appearance is retained in suspension when the heat treatment is adequate.

Both citrus processors and can manufacturers have shown a great deal of interest in this test, termed an index of pasteurization, which is proving extremely valuable as a control measure in canning juices.

Other studies, in a less advanced stage, show a definite relationship between the extent of pasteurization and the keeping quality of canned orange juice. A taste panel, for example, could distinguish juices that had been subjected to pasteurization for 40 seconds, 13 seconds, or 1½ seconds when they were served after having been stored for 6 months at a temperature of 70° F. Some differences in the composition of fresh pasteurized and unpasteurized juices have been noted, and further changes in composition develop as the juice ages. Studies are being continued to enlarge upon the knowledge acquired thus far and to translate the data to practical use.

Effect of Storage Temperatures on Frozen Citrus Concentrates Studied

Studies that will aid processors and handlers in the storage of frozen citrus concentrates have been undertaken in cooperative work under an RMA project by the Citrus Products Station at Winter Haven, Fla., and a commercial processor of citrus fruits.

The presence of some well-distributed pulp and cloud in citrus juices is considered essential for both flavor and appearance, and any factors tending to cause instability in these attributes are considered undesirable. Stability can be attained by maintaining the product at a sufficiently low temperature. No significant changes were noted in cloud or flavor during 11 months' storage at 0° F. or below. At 5° F., change in cloud retention was slow but definite. At progressively higher temperatures, changes in cloud and flavor took place at progressively higher rates.

Probably the effects of storage at temperatures above 0° F. are cumulative and irreversible, and the effects of time and temperature must be considered whether it be in warehouses, during transportation, or in retail and consumer channels.

Safe Storage Conditions for Citrus-Pulp-and-Molasses Feed Defined

In the processing of citrus fruit for juice or frozen concentrate, two important byproducts are produced. These are citrus pulp, a dried material used as a feed, and citrus molasses, obtained from the liquid residue. The molasses is a thick liquid and very difficult for the dairyman or cattleman to handle. By drying the molasses with the pulp, a feed of uniform quality, easy to handle, is obtained.

However, certain questions arose in connection with this procedure. First, the industry needed to know how the addition of citrus molasses would affect the keeping of the citrus pulp. Also, because the addition of molasses increased the soluble-solids content of the dried feed, a method of analysis was needed so that a product of uniform composition and feeding value could be produced.

In an effort to answer these questions, the Citrus Products Station in Winter Haven, Fla., has made a thorough study of citrus pulp enriched with citrus molasses.

The addition of citrus molasses did not introduce any serious storage problem. Lots with added molasses stored practically as well as those without. The studies revealed also that no difficulties would be expected in storing these feeds at relative humidities of 70 percent or less, but at 80 percent relative humidity the samples became moist enough to mold and spoil.

The Citrus Products Station also developed a method for estimating the soluble-solids content of feeds made from citrus pulp. Dried citrus feeds have always contained considerable soluble solids, the amount varying somewhat with the process used, condition of the fruit, and other factors. The addition of citrus molasses increases the soluble-solids content, and, with proper factory control by using the method developed, a more uniform product can be produced. The method is simple and rapid, yet is accurate enough for routine control work. Feed mills are using it to advantage in their operations.

Nitrogenous Components of Citrus Juices Identified

The darkening and development of off-flavors during the processing and storage of citrus-juice products materially reduces their quality. Citrus-fruit processors have suspected for many years that unknown nitrogenous compounds in citrus juices may contribute to the unfavorable reactions that result in loss of the characteristic properties of fresh citrus juices. It is well known also that amino acids and other organic nitrogen compounds react with sugars, such as are known to be present in citrus juices, with the formation of unusual colors, flavors, and odors.

Little work has been done on the determination of the nature, amounts, and reactivities of the nitrogenous substances that compose almost 10 percent of the solids in citrus juices, principally because of the unavailability of suitable analytical methods.

During the past few years several new methods have been elaborated for the determination of amino acids and other nitrogenous substances. One of the most rapid and convenient of these—partition chromatography—may be applied to the qualitative and quantitative estimation of many types of compounds in complex mixtures. The Fruit and Vegetable Chemistry Laboratory at Pasadena, Calif., has developed, under an RMA project, a series of rapid, small-scale filter-paper partition chromatography techniques that may be applied to the analysis of nitrogenous and other constituents in fruit and vegetable juices and extracts.

Chromatographic techniques have been applied to the qualitative analysis of nitrogenous compounds in fresh and processed Valencia and navel oranges and in grapefruit, lemon, tangerine, and lime juices extracted from California, Florida, and Texas fruits. It was found that the organic nitrogen in citrus juices is present largely in the form of free amino acids and other simple molecules. The various citrus juices contained the amino acids alanine, aspartic acid, asparagine, glutamic acid, and serine in amounts approximating the vitamin-C content of the juices. In addition, Valencia orange juice contained

arginine, proline, and another major constituent believed to be sarcosine. Proline, but neither arginine nor sarcosine, was found in navel-orange juice. Grapefruit and tangerine juices were identical in respect to their major nitrogenous constituents, containing arginine in addition to the five amino acids found in all the juices. Lemon juice contained only the five amino acids. Among the important minor nitrogenous constituents that have been identified in citrus juices are cysteine and glutathione. The Enzyme Research Division in the Western Regional Laboratory previously reported the identification of these compounds in orange juice. Quantitative filter-paper-partition chromatography studies of fresh and heat-treated orange, lemon, grapefruit, and lime juices show that all contain small but significant amounts of both cysteine and glutathione, and that about 25 percent of each of these compounds is destroyed by heating. The foreign flavors and aroma developed in processed citrus products may be due in part to the formation, from these two sulfur-containing compounds, of hydrogen sulfide and other sulfur-containing decomposition products.

The presence of relatively large amounts of free amino acids in citrus juices indicates that the role of these compounds in deteriorative changes of processed fruit juices may be even more important than previously suspected. This is particularly true in concentrated juices where more nearly optimum conditions are provided for the Maillard (browning) reaction between proteins and sugars. Further work must be done to determine the relationship between individual nitrogenous constituents and the deterioration of citrus-juice products.

Molecular Constitution of Pectin Explored

Pectin is the carbohydrate familiar as a jellying agent in fruit jellies, jams, and marmalades. It is present in practically all of the higher plants, being particularly prominent in the peel of citrus fruit, in apple pomace, and in sugar beets. Because of its properties and location in the intercellular tissue, it plays an important part in controlling the texture of many fruits and vegetables. Furthermore, the amount of pectin available for commercial production and use is considerable—nearly 100,000 tons in peel from citrus processing alone.

The scientist knows pectin as a polymer of galacturonic acid, a simple sugar in which the end group is an acid instead of the usual alcohol. About 75 percent of the acid groups in pectin are esterified with methyl alcohol. In addition, there is between 10 and 40 percent of material that is neither uronic acid nor its methyl ester. Scientists have long been puzzled by this nonuronide and have wondered about its identity and whether it is part of the pectin molecule or merely associated as a ballast material. Because of its possible contribution to the chemical and physical properties of pectin, these questions have been studied at the Western Regional Laboratory.

By application of chromatography, X-rays, and crystallography, the nonuronide was found to be composed of arabinose, galactose, and rhamnose. Rhamnose had not been previously identified in pectin preparations.

In agreement with results obtained at other laboratories, it was found that precipitation of pectin from water solution with alcohol or copper salts did not remove the nonuronide. Even deesterification of

the pectin, followed by extraction of the resulting pectic acid with boiling water, did not remove the contaminant. Extraction of pectin with hot 70-percent methyl alcohol removed some of the nonuronide but not in sufficient quantity to be conclusive. Application of a direct electric current to pectin solutions caused the pectin to move to the anode. This pectin lost some araban but most of the nonuronide went along, even though it possessed no charged group. All this evidence favors the hypothesis that nonuronide is part of the pectin molecule.

If the pectin is deesterified by alkali, precipitated several times by acid, and then subjected to a direct electric current, a fairly pure polygalacturonide concentrates in the anode chamber. Also, if pectin is esterified with propionic anhydride and then deesterified with alkai, most, if not all, of the nonuronide is removed. These results lead to the hypothesis that the nonuronide is held to pectin either by ester groups as part of the molecule or by hydrogen bonds.

The significance of the findings lies in the fact that for all studies of pectin in tissue, such as problems of rehydration of dehydrated fruit, extraction of pectin for commercial uses, and formation of pectin jellies, the nonuronide material must be taken into account as though it were a part of the pectin molecule.

More Learned About Browning of Cut Fruits

An important problem in freezing preservation of some cut fruits, such as apples, apricots, and peaches, is the browning that appears on their surfaces, especially after the frozen fruits are thawed. Besides detracting from appearance, the browning reduces the nutritive value of the food because it is accompanied by loss of vitamin C.

No wholly satisfactory methods of preventing this deterioration are known. Treatments with certain chemicals are costly, prevent the browning for only a limited time, or are undesirable for other reasons. Blanching (scalding) is effective for prevention of browning, but it causes undesirable softening of texture and leaching of nutrients. Although it is known that one type of browning is brought about by a fruit enzyme (polyphenolase) in the reaction between oxygen of the air and tannins, very little information is available on the chemical identity of tannins in various fruits, about the characteristics of the enzyme, about the chemical nature of the brown products, or about the chemical steps that lead to browning. It is evident that further advances in control of enzymatic browning depend in large measure on greatly increased knowledge along these lines.

Investigations at the Western Regional Laboratory contributed to an understanding of the browning problem.

A histochemical test was developed for determining the qualitative distribution of the tannins within the fruit tissues. This will give an indication as to which portions of the fruit are most susceptible to browning.

In experiments on isolation and identification of the tannins in peaches (conducted under an RMA project) modern techniques, such as chromatography and countercurrent extraction, led to a positive identification of caffeic acid as an important browning substrate. This information should be useful in further studies on the mechanism of browning. It might also prove valuable to plant breeders in the development of a chemical method for rapid testing

of the browning susceptibility of new strains of fruits in the seedling stage.

As chlorogenic acid has been reported as a likely browning substrate in fruits, the rate of its nonenzymatic oxidation by oxygen has been investigated. The initial rate was found to be directly proportional to the concentration of chlorogenic acid and pressure of oxygen, and inversely proportional to the concentration of hydrogen ion. The rate law has been interpreted in terms of a mechanism that postulates the formation of a semiquinone as an intermediate. The results will make it possible to interpret the contribution of nonenzymatic oxidation in later studies of the enzymatic oxidation of the tannins.

A study has also been made of the role of ascorbic acid (vitamin C) in the enzymatic oxidation of catechol. Ascorbic acid reacts with oxidation products of the catechol and thus inhibits the browning until all the ascorbic acid has been destroyed. During this process some of the enzyme also is destroyed. When an excess of ascorbic acid is used, the amount that is eventually oxidized is a function of the amount of the enzyme initially present. Previous investigators (using mushroom polyphenolase) have reported that the rate of the enzyme destruction is "three-halves order" with respect to the enzyme concentration; they have interpreted this in terms of a very complex series of reactions involving a gradual enzyme degradation. The new results (with apple enzyme) show a "first-order" rate dependence and have been interpreted in terms of a much simpler and plausible mechanism that postulates the destruction of the enzyme by a semiquinone-free radical. They may lead to the development of a simpler method of measuring polyphenolase activity than has been used thus far.

Study Made of Heating Conditions Required to Prevent Browning of Fruit Purees

Many freshly prepared fruit purees are subject to a discoloration (browning) that is brought about by the oxidation of tannins in them through the agency of an enzyme called polyphenolase. This discoloration, which is accompanied by an undesirable change in flavor and loss of vitamin C, is particularly rapid and troublesome when frozen purees are thawed. Development of improved methods for the prevention of this degradation is one of the important problems in the fruit-processing industry.

A valuable contribution to the solution of this problem has been made at the Western Regional Laboratory through an investigation of the rate of destruction of polyphenolase in several fruit purees at different temperatures. Although it was previously known that sufficient heating will destroy the enzyme and prevent the discoloration, very little information was available on the specific time and temperature conditions required for the treatment. Rigorous control of the processing conditions is important. Overheating will destroy the natural fresh-fruit flavors; underheating will result in incomplete destruction of the enzyme, which, even in small amounts, causes subsequent darkening of the puree.

From the rate measurements made on purees of grape, peach, apricot, apple, and pear it was possible to estimate the time and temperature conditions for essentially complete inactivation of the polyphenolase. It was found that different conditions are required for the different

fruits. For a given fruit, the required heating time for inactivation increases very markedly for a small decrease in temperature. This is in accord with the behavior of most proteins toward heat. It was also found that the inactivation time is greatly affected by changes in hydrogen-ion concentration (pH) and that each fruit can be characterized by a pH of maximum enzyme stability.

These results, which were obtained with certain selected samples of fruits, will serve as guides for further research. It is also evident that fruits of different variety or maturity, or from different localities, may require somewhat different heat treatments because of variations in composition.

The work is being continued to determine the effect of these variables on the inactivation requirements for several commercially important fruits.

Volatile Components of Strawberries Identified

One of the important problems in processing fruits is the preservation of the natural aroma. Because much of the aroma is lost by volatilization in various processing operations, especially in cooking, an effort is being made in Bureau laboratories to develop methods whereby the volatile flavoring substances would be recovered from the fresh fruit, concentrated to an essence, and later returned to the product of processing.

The recovery and concentration of the volatile flavors present several technical problems, the solution of which would be greatly aided by knowledge of the chemical identity and amounts of the volatile flavoring constituents. These problems include quantitative determination of the strength of the essence, determination of the distillation characteristics of the essence, and evaluation of different varieties of a given fruit as to their flavor intensity and suitability for processing. Another important problem is the development of a method for determining the ethyl alcohol content of the essence. This is needed for control purposes and for compliance with regulations set up by the Bureau of Internal Revenue. Some of the information sought will be of interest to plant breeders in development of more flavorful strains of fruits, as well as to pomologists in studies of the effect of the various volatile substances on the ripening and storage stability of the fruit.

Investigations at the Western Regional Laboratory on the preparation of strawberry essence and analysis of the volatile constituents have contributed much of the desired information. These studies, a part of an RMA project, were made in cooperation with the Western Washington Agricultural Experiment Station on several varieties of strawberries grown in the Pacific Northwest.

A quantitative method was developed for estimating the total amount of volatile organic material removed from the fruit by distillation. With this method it was found that approximately 50 percent of the water must be flash-vaporized from a strawberry puree to recover about 90 percent of the flavor. It was shown further that excessive heating of the aqueous distillate causes flavor degradation; this precludes concentration of the flavor by batch distillation. Distillation in a continuous still, however, is satisfactory. It was also found that a homogeneous strawberry concentrate cannot be prepared in strength greater than about three-hundred-fold, because of the formation of two immiscible liquid layers.

Flavor evaluation of seven varieties of northwestern strawberries showed that they vary markedly in flavor intensity. That this variation is probably characteristic of the varieties is indicated by the observation that approximately the same order of intensity for the different varieties was found for two different harvest seasons.

Chemical identification of the volatile organic components was carried out on the essence of Marshall strawberries, the most important commercial variety in the Northwest. About 90 percent of the organic material was found to consist of biacetyl, acetaldehyde, acetone, 2-hexenal, methyl alcohol, ethyl alcohol, several acids, and some unidentified esters. About 50 percent of the nonaqueous material was ethyl alcohol.

A strongly aromatic fraction, comprising about 10 percent of the organic material, still remains to be identified. The work is being continued and will be expanded to include a study on preparation of high-strength concentrates and on determination of the factors that govern their stability, especially in highly concentrated fruit products such as are needed in connection with the defense effort.

Frozen Juice Concentrate From Western Apples Evaluated

Washington State apple growers have joined with the Western Regional Laboratory and the Bureau of Agricultural Economics and the Washington State Agricultural Experiment Station for the purpose of determining the feasibility of manufacturing an acceptable frozen apple-juice concentrate from the varieties of apples grown in the State of Washington, with these varieties used in the proportions, qualities, and grades in which they become available for commercial processing. This cooperative enterprise involved process development and consumer-preference and market tests of the product.

The first step was to determine the varietal blend of apples to be used in preparing the market-test samples of frozen apple-juice concentrate. The possibilities included various combinations of Standard Delicious, Winesap, Jonathan, Rome Beauty, and Golden Delicious. All blends tested contained at least 50 percent Delicious apple juice, roughly the proportion of this variety in the Washington apple crop. Through use of discrimination and preference tests, carried out by the Washington State Agricultural Experiment Station, the number of possibilities was narrowed down to three, representing the top quality of frozen apple-juice concentrate obtainable from the raw material. The three categories (straight Delicious, acidified Delicious, and a blend of Delicious, Winesap, Jonathan, and Rome Beauty mixed in the ratio 5:2:2:1) of frozen apple-juice concentrate were then submitted to a consumer-preference test, carried out by the Bureau of Agricultural Economics in the San Francisco Bay metropolitan area. The consumer-preference test showed definite preference for the blend and the acidified Delicious over the straight Delicious juice. There was little difference in choice between the blend and the acidified Delicious.

Upon completion of the consumer-preference tests, about 40,000 6-ounce cans of fourfold frozen apple-juice concentrate was prepared by the Western Regional Laboratory, as part of an RMA project, for use in the market test. The composition of this juice was similar to that of the blend used in the consumer-preference test.

The method used for preparing the concentrate was described in the 1950 Report of this Bureau. The volatile flavor or essence is stripped from fresh juice, and the stripped juice is concentrated to slightly over fourfold. The essence is returned to this concentrate to produce a fourfold product, which is immediately frozen and stored and handled as a frozen product. The process of stripping the juice and concentrating the essence has been described in publications by the Eastern Laboratory. A modification of the process, involving use of a steam-injection heater, which was developed by the Western Laboratory, was used in preparing the concentrate for these studies. The stripped juice was concentrated in a vacuum pan. The Western Laboratory has shown that this step can be carried out without impairment of quality at temperatures as high as 130° F. Thus, less expensive equipment may be used for concentration of apple juice than for orange juice, where evaporation temperatures below 85° F. are ordinarily used.

The market test on frozen apple-juice concentrate was conducted cooperatively by the Washington State Apple Commission and the Bureau of Agricultural Economics.

In this venture the efforts of the Washington State apple industry were coordinated by the Industry Apple Juice Concentrate Committee appointed by the Washington State Horticultural Association. This committee has evaluated the results of the whole project and has drawn definite conclusions for guidance of the industry's future action.

Full-Flavor, Superconcentrated Fruit Juices Keep in Ordinary Cold Storage

Storage tests on high-density, full-flavor apple- and grape-juice concentrates (approximately 70° Brix) at the Eastern Regional Laboratory pointed to the general conclusion that such concentrates can be kept satisfactorily in ordinary cold storage. When diluted with water after a year's storage, they still formed juices comparable in quality with high-grade commercial bottled juices. The fact that they do not have to be frozen and kept in freezing storage greatly extends the field of distribution of such fruit concentrates, even to overseas shipment where freezing storage is not available. Warehousing and shipping costs are considerably less than for ordinary frozen concentrate, first, because maintenance of near-zero temperature is not required and, second, because less weight of the superconcentrate is required to produce the same quantity of juice of drinking strength. One can of high-density concentrate will make seven cans of juice, whereas only four cans are obtained from a can of frozen concentrate. It thus should be of particular utility and economy for large-scale use and long-distance shipping.

In the case of apple concentrates, the juice from the seven-fold product resembles a high-grade clarified single-strength processed juice, whereas a fourfold frozen product yields a beverage closer to a freshly pressed juice. The choice is one of personal preference.

The superconcentrates are made by methods already published by the Eastern Laboratory. Fresh apple juice, expressed in the usual way, or stored grape juice of the usual concentration is passed with extreme rapidity through the essence apparatus, where the volatile

flavor is stripped off and concentrated to form an aqueous essence. The stripped juice is next depectinized with an enzyme preparation, so that it may be concentrated to high density without gelling. It is then filtered, and, after inactivation of the enzyme, is concentrated to about 70° Brix. After the essence is added to the concentrate, the mixture is cooled and packed in cans of the desired size (4-ounce or larger). For a satisfactory product, the fruit must be of good quality, sound, and mature, and the juice must be handled in a careful, sanitary manner.

New Fruit Essences in Prospect for Improving Preserves or Use as Flavors

Work was done at the Eastern Regional Laboratory on the development of a method for the recovery of volatile flavor normally lost in the customary vacuum-evaporation process of making fruit preserves. The flavor is recovered as a concentrated aqueous essence, which may either be returned to the preserves or sold for other flavoring purposes. With the fruits thus far tested (cherry, peach, strawberry, and blackberry) good recovery of the flavor has been attained.

The usual process of making preserves commercially consists of mixing the fruit with sugar and pectin and concentrating the batch in a vacuum pan to 65°–68° Brix. To apply the essence-recovery method, it is necessary to have a surface condenser on the vacuum pan; if an existing pan has a water-jet or barometric condenser, a surface condenser must replace it. An essence-recovery apparatus similar to the one previously used on apple juice, as described in publications of the Eastern Laboratory, is also required. The condensate from the surface condenser, which usually amounts to 16 to 18 percent of the starting batch, is sent to the essence apparatus, where the fragrant substances are stripped from it and concentrated to form an aqueous essence. With both cherry and peach condensates, practically complete stripping was obtained by vaporizing 20 percent of the original condensate in the essence-recovery apparatus. For strawberries and blackberries, 40-percent and 30-percent vaporization, respectively, was required.

The water supplied to the surface condenser should be cold enough to cool the condensate to 65° F. If it is not that cold, a small scrubbing tower can be used in the air line between the condenser and the vacuum pump for more complete recovery of volatile flavor.

If the essence is returned to the preserves, their flavor and aroma will be stronger and more characteristic of the fruit. If, however, the flavor of the preserves as ordinarily made is considered sufficiently characteristic and strong, as is usual with cherry preserves, the essence itself should find a ready market as flavoring for other food products such as candies, carbonated beverages, and ice cream.

New Frozen Spread Made From Avocados

The avocado is not produced in this country in oversupply at present, although yearly increases in plantings in southern California are being made, and surpluses in the near future may create a marketing problem. Therefore studies were carried out at the Fruit and Vege-

table Chemistry Laboratory, in Pasadena, Calif., to develop new processed avocado products which may add to the variety of the American diet and also serve as outlets for surplus and cull fruit.

Preservation of the avocado has been considered a difficult problem for many years. Attempts have been made to preserve avocado slices and halves by methods requiring heating or freezing. These attempts were never successful because of the extreme ease and rapidity with which the tissues of the fruit turn brown on exposure to air or during heating. Avocado products, such as sandwich spread and salad dressing mixtures, as well as avocado slices and halves preserved by pickling, have been marketed in small quantities.

"Guacamole," a popular avocado spread, is made from pureed avocados, salt, and lemon or lime juice, flavored with onion or garlic. This spread retains to a high degree the desirable, unique flavor of the fruit, but it turns brown in 4 to 8 hours after preparation, even when refrigerated. Experiments were conducted to improve the color retention of this product by varying the proportions of ingredients used in the recipe. As a result, the formula was developed for preparing an avocado paste that retains attractive green color without darkening for at least a year in frozen storage, and for 1 to 2 weeks after thawing and storage at refrigerator temperatures. This has been accomplished primarily by adding larger proportions of lemon or lime juice and salt to the avocado puree. Avocado paste can be manufactured from overripe or second-grade fruit; when properly handled, a desirable product, suitable for distribution as a frozen food, is obtained.

The method developed for the preparation of frozen avocado paste is comparatively simple. Sound, ripe fruit is thoroughly washed, preferably with a good detergent, and rinsed well with cold water so as to reduce microbial contamination to a minimum. The fruit is pared, and all discolored spots, damaged portions, and the seed are removed. The fruit is then pureed by sieving or passing through a food grinder. Sieving gives the product a smoother texture, but many persons prefer the coarser texture produced by grinding. The avocado puree is then mixed with lemon or lime juice, salt, and onion powder in the following proportions (by weight): Avocado puree, 100 parts; lemon or lime juice, 8.0 to 10.0 parts; salt (sodium chloride), 1.0 to 2.0 parts; and dehydrated onion powder, 0.3 part. The ingredients are blended, and the finished product is put into a suitable container and frozen at 0° to −10° F. The quantity of onion powder may be varied according to taste, or the onion powder may be omitted. Garlic powder may be used to vary the flavor of the product.

Glass, enamel-lined tin, plastic, and wax-impregnated fiber containers are suitable for packaging the avocado spread. However, the most novel and convenient type of container for the product is a collapsible tube of aluminum or tin foil. With this type of container, the withdrawal of small portions of the thawed product as consumed leaves no headspace for contact with air which might accelerate discoloration. Avocado paste prepared and packaged as described has been found to retain satisfactory color and flavor after storage for 1 year at 0° to −10° F.

Advances Made in Utilization of Fruit-Cannery Wastes

Further progress has been made in the development of practical processes for the utilization of fruit-cannery wastes. The work is divided into two separate and somewhat distinct phases, one dealing with the processing of gross cannery wastes for the production of feed and other nonedible products, the other with the processing of selected cannery waste for the production of an edible sirup suitable for return to the canning line to replace part of the sugar normally used.

Feed and molasses from fruit wastes

Pilot-plant studies were continued under an RMA project during the 1950 season in cooperation with the Canners League of California. The League, an organization representing most of the fruit canners in California, supplied plant facilities and financial support for the project. The Cling Peach Advisory Board also assisted in financing the project.

The 1950 operations were guided by the results obtained in pilot-plant studies the previous season at San Jose, Calif., and in process studies on a laboratory scale in the winter of 1949–50. The laboratory studies led to two important developments. One was a greatly improved process for treating pear waste to facilitate separation of juice from pomace, and the other was a new type of dejuicing machine for separating juice from pomace. Both were applied successfully on a pilot-plant scale during the period of August 14 to October 16, 1950.

The new treating process proved to be relatively simple in use and highly flexible. Liming of the ground pear waste under controlled conditions, in a two-stage process, converts it from a slimy mass to a form suitable for separation into juice and pomace fractions. The process works satisfactorily on wastes from pears of all varieties and maturities, as well as on mixed wastes containing pear, peach, grape, and tomato wastes. The new dejuicing machine greatly facilitates the processing by combining dejuicing of the waste and pressing of the cake in a single, continuous, easily controlled operation. Commercial equipment previously used required separate machines for dejuicing the waste and pressing the pomace.

About 140 tons of pear waste was processed during the season. The pear molasses and dry pear pomace were used in stock-feeding and other utilization tests. Feeding tests made by the California Agricultural Experiment Station showed pear molasses and pear pomace containing 20 percent pear molasses to be very palatable to sheep and cattle and indicated that these products should find a ready local market as feedstuffs. Tests by the Bureau of Entomology and Plant Quarantine showed that pear pomace as a carrier for insecticide in grasshopper baits of the dry type was somewhat better than dry bran.

This development promises to be another major contribution to the field of fruit-waste utilization. Operation during the canning period of 1951 has proceeded so well that the research phase of the project should be substantially complete at the end of the season.

Sirup from pear waste

A process for the recovery of sugars from pear-canning waste was operated on a semicontinuous pilot-plant scale in the 1950 pear-can-

ning season by the Fruit and Vegetable Products Laboratory in Prosser, Wash., under an RMA project. The process, mentioned in last year's report, recovers sugars and other soluble solids from peeling and cores in the form of a clear, colorless juice that the canner may use as a sirup base in canning pears. The sugars recovered by this process would replace about one-third of the refined sugar normally used in canning the fruit.

The pilot-plant operation was conducted in cooperation with the Apple Growers Association, a farm cooperative in Hood River, Oreg., in the association's cannery. The technical practicability of the process was demonstrated, and operational details of the process were worked out for use in commercial applications of the process. During the course of the study, 2,500 gallons of juice was refined and used in preparing 1,500 cases of canned pears for consumer acceptance tests by the association. Pears canned in juice sirup are considered equal in appearance and flavor to pears canned in the ordinary sugar sirup.

POULTRY PRODUCTS

Rancidity in Precooked Frozen Turkey Retarded

Rancidity develops to a significant degree in frozen cooked-turkey products, such as turkey à la king. The rancidity may develop during preparation and also during frozen storage. The use of antioxidants as a means of preventing or retarding rancidity in turkey products has been studied at the Western Regional Laboratory under an RMA project.

Although the use of nontoxic antioxidants to retard rancidity development is common practice, there is relatively little information on their use during cooking of foods such as turkey. Experimental use of a small amount of a food-grade antioxidant in the cooking water has produced cooked turkey with no detectable rancidity immediately after cooking and little tendency to develop rancidity during frozen storage as creamed turkey or turkey à la king. The turkey fat rendered during cooking was used in the creamed turkey, and, unlike fat recovered in the absence of antioxidant, it appeared stable enough to be used in other food products also.

The turkeys used in the experiments were cooked by simmering in water in a covered container, antioxidant being added to the water at the beginning of the cooking process. Half of each turkey was cooked with antioxidant; the other half without. The cooked turkey meat was removed from the bone, diced, and added to sauces prepared from turkey fat, broth, flour, and milk. Comparisons were also made with samples of creamed turkey to which the antioxidant was added after the turkeys were cooked.

Development of rancidity during cooking of the turkey and during storage as frozen creamed turkey was proved by peroxide determination (a recognized index of rancidity) and by tasting. In one series of experiments, peroxide values for turkey cooked without antioxidant ranged from 8 to 43, whereas samples cooked with antioxidant had peroxide values ranging from 1 to 4. The variation in peroxide value of turkey cooked without antioxidant appears to be due to inherent differences in the birds. A rancid flavor was not detected in the creamed turkey prepared from the halves cooked with the anti-

oxidant, but with some lots of birds creamed turkey prepared from the halves cooked without antioxidant tasted rancid even before it was subjected to frozen storage.

The development of rancidity is also retarded when antioxidant is added during the preparation of the sauce for the creamed turkey, but it appears more advantageous to add antioxidant during cooking of the bird in order to prevent the initiation of rancidity that otherwise occurs. An antioxidant mixture composed of 20 percent butylated hydroxyanisole, 6 percent propylgallate, 4 percent citric acid, and 70 percent propylene glycol was used in most of this work. It was ordinarily added to the cooking water at a level of 0.005 percent based on the weight of the bird. Creamed turkey stored in ordinary packaging material for 12 months at 0° F. showed no significant increases in rancidity as determined by either flavor score or peroxide value when antioxidant was present, but did show significant increases by both tests when no antioxidant was present.

Possible Causes of Egg Deterioration Shown by Effects of Chemicals

Shell eggs rapidly lose quality unless extensive precautions are taken to preserve their initial freshness. Among the changes that occur in storage are loss in weight and some changes in physical structure due to evaporation of moisture. This deterioration can be retarded by prevention of the loss of moisture. Two other important changes are the breakdown and thinning of the egg white and the weakening, flattening, and rupture of the yolk. The causes of these changes are unknown, although a number of factors, such as temperature of storage and oiling of the shell, are known to influence the rates at which they occur. If the causes were known, it should be possible to develop more adequate processing methods for the control of such deterioration. One approach toward determining the chemical reactions involved is to study the effect of specific chemical reagents on eggs and to compare the changes caused by the chemicals with changes that occur naturally in storage.

Recent studies at the Western Regional Laboratory, continuing the Bureau's research on the chemistry of eggs, have had striking results by this approach. Changes resembling natural deterioration have been obtained by the addition of minute amounts of any one of several chemical reducing agents. Depending on the amount of reagent added, the manner of its administration, and the time of action, varying degrees of thinning of the white and weakening of the yolk were obtained that were similar to the varying degrees of such changes found during natural deterioration. Among the reducing agents that will effect these changes are hydrogen sulfide, sulfur dioxide, thioglycol, thioglycollic acid, cysteine, and hydrogen cyanide.

The action of the reducing chemical was shown to take place on the membrane of the yolk and on the ovomucin fraction of the proteins of egg white (considered to be responsible for the jellylike nature of thick egg white). It is probably the same as the action taking place on certain other fibrous-type proteins. Disulfide bonds in the structure of the proteins are broken when the materials are treated with reducing chemicals, yielding smaller, but still comparatively large, particles and losing their fibrous structures. Although these studies have not shown that the deterioration of the white and yolk is actu-

ally caused by chemical reducing agents formed or already present within the egg, the demonstration that such agents can cause similar changes is important. Of importance is the observation that the changes in the yolk and white are both brought about by the same agent, which probably means that the deterioration of the yolk and that of the white during storage have the same cause and mechanism. If the disulfide bonds in certain egg proteins can be split by reducing chemicals during storage, they can probably be split also by other means. For example, it is known that disulfide bonds are slowly attacked by alkali. Finally, chemical reduction itself may be involved in the deterioration during storage. Reducing groups actually exist in comparatively large amounts in egg white, but they are masked or not very reactive.

The experiments with added chemicals showed also that absorption of reducing chemicals though the shell might cause deterioration of eggs. Deterioration was actually found when eggs were stored in atmospheres containing very low concentrations of hydrogen sulfide and sulfur dioxide. Oiling the egg, as is done commercially for eggs going into cold storage, effectively retarded this absorption. Thus, the practice of oiling, long employed to reduce deterioration by keeping volatile materials (water and carbon dioxide) from leaving the egg, may be equally useful by preventing absorption or adsorption of reducing gases by the egg. However, the economic importance of deterioration caused by exposure to reducing gases remains to be investigated. A critical survey is needed of the concentrations and amounts of reducing chemicals to which eggs are exposed during handling and storage.

New Results Obtained on Food Poisoning by Salmonella Bacteria

Improvements in processing methods during recent years have resulted in the production of better and safer foods from the microbiological standpoint. The possibility of food poisoning by salmonella organisms has been known for many years, but there are so many facets to the problem of eliminating them that the extensive work done thus far has not supplied all the information needed to appraise the danger in various foods and to develop practical means for eliminating or minimizing the bacteria. There are more than 150 recognized species or types of salmonella, at least 50 of which have been implicated specifically in food-poisoning outbreaks. Actually all species of salmonella are by definition pathogenic for either animals or man or for both. It is presumed that the remaining 100 species are pathogenic for man.

Studies of the pathogenicity of salmonella and further characterization of the clinical aspects of illness resulting from oral feeding of known numbers of salmonella have been conducted at the University of Chicago under an RMA contract supervised by the Western Regional Laboratory. Twelve strains representing six species were used. All but one of the strains had been isolated from dried whole egg and were subsequently preserved in the dry state to avoid any possibility that their virulence might change because of repeated subculturing on artificial media. They were: *S. pullorum*, *S. meleagridis*, *S. anatum*, *S. newport*, *S. derby*, and *S. bareilly*. As all of these species (as isolated from various sources) had been implicated in food-

poisoning outbreaks, it was expected that they would be found to be pathogenic at some dosage level. The study has provided information on the dosage levels for oral feeding that cause overt illness and has provided information on the clinical manifestation of illness arising from oral feeding of food containing salmonella.

The study was conducted with human volunteers in a penal institution. Various numbers of viable salmonella organisms were placed in eggnog and fed to the volunteers, who were subsequently observed for manifestation of illness. Suitable control feedings were part of the experimental design. Generally six men were fed at any selected dosage. The lowest dosage (number of micro-organisms) causing overt illness of at least one man in six was 125,000. The numbers required to cause illness varied with the species and the strain. In one case as many as 1.3 billion micro-organisms per dose were required to cause one man in six to become ill. As the number causing illness varies with the strain as well as with the species and undoubtedly with the age and state of health of the subject, the number limits must be considered illustrative rather than definite. Several volunteers became carriers, even though no illness was apparent after the feeding. All cases of illness were followed by recovery, and all carriers became negative after several months.

In spite of the rather widespread occurrence of salmonella, citations of salmonella as a cause of illness are fairly limited. Perhaps this reflects the general rule that many cases of food poisoning go unreported if the illness is mild, as it may be in many cases when salmonella-infected food is eaten.

Nevertheless, this study of the detailed pathogenic characteristics of twelve strains of salmonella, when considered along with information available from other sources, emphasizes the view that more attention should be given to the control or elimination of salmonella organisms during the processing and preparation of food, particularly animal products. This Bureau, other research institutions, and industry are intensifying research activities on methods for reducing the occurrence of salmonella in foods.

MISCELLANEOUS FOOD PRODUCTS

More Stable Sauces and Gravies Made for Use in Precooked Frozen Foods

Sauces and gravies markedly increase the flavor stability of precooked meat and vegetable dishes by replacing the air in the package. Yet the separation ("weeping" and curdled appearance) that follows the thawing of sauces and gravies thickened with wheat flour after freezing or frozen storage is generally considered a defect that reduces consumer acceptance of such products as chicken á la king. Methods of preventing this separation by using other starches, flours, and commercial stabilizers have been studied under an RMA project at the Western Regional Laboratory.

Tests have shown some improvement in the stability of the sauces with several out of about 60 products. Of these waxy (glutinous) rice flour was outstanding in all respects. In flavor and appearance, unfrozen sauces prepared with waxy rice flour are difficult to distinguish from those prepared with wheat flour. Sauces thickened with

the waxy rice flour could not be distinguished from the freshly prepared unfrozen product after storage for more than 10 months at 0° F. But with wheat flour, the separation was apparent even when the product was thawed immediately after freezing, and it became progressively more pronounced with prolonged storage.

The investigation has revealed that the type of thickening agent is the principal factor in the development of a curdled appearance and separation of liquid in sauces and gravies subjected to freezing and frozen storage. With starches and flours high in amylopectin these defects are at a minimum. As amylopectin starches, in contrast with the waxy cereal flours generally, yield a "long" (tenacious) paste, however, the starches themselves do not appear suitable for use in sauces and gravies. Flours from waxy corn and grain sorghum yield sauces having improved stability, although the improvement is not quite as great in some respects as that obtained with waxy rice flour. Waxy rice flour has given the best results in the tests made thus far.

The temperature to which sauce is heated after being frozen influences the appearance and liquid separation. Ordinary sauces thawed at room temperature shortly after being frozen show marked curdling and liquid separation, and these defects are more pronounced after frozen storage. Heating of thawed sauces made with ordinary flour does not completely eliminate the appearance defect and liquid separation, although both of these factors are decreased. Even frozen sauces made with the waxy cereal flours may show considerable liquid separation when they are thawed at room temperature after being subjected to extended frozen storage (particularly if the storage temperatures are as high as 10° to 20° F.). However, when these sauces are heated, the liquid is completely taken up by the opaque phase, even without stirring, and a perfectly smooth sauce results. The defects in appearance of sauces and gravies can be reduced by using mixtures of wheat flour and waxy rice flour, particularly when the product is homogenized before freezing.

Method Devised for Counting Spores of Food-Poisoning Bacterium

The most important food-poisoning organism from the viewpoint of the processor of vegetables and other nonacid foods is *Clostridium botulinum*. Because it produces an extremely potent and deadly toxin, and because its spores are very resistant to heat, commercial canning processes are largely based on the destruction of this bacterium or of the similar but somewhat more heat-resistant test organism, Putrefactive Anaerobe NCA 3679. Because of its importance in public health, extensive studies have been made of *C. botulinum*, but these studies have been hampered by the lack of an adequate method for counting the spores.

In order to count the number of viable spores in a sample, it is necessary that they germinate and form colonies, but *C. botulinum* exhibits dormancy to a marked degree; that is, germination of many of the spores is delayed when suspended in ordinary media. For this reason workers have had to resort to incubation periods of many weeks and even then have often obtained low spore counts. Several bacteriologists have observed that carbon dioxide is beneficial to the growth of *C. botulinum*, but apparently this fact has not been used to improve counting methods.

A procedure developed by the Western Regional Laboratory makes possible accurate spore counts after only 24 to 40 hours' incubation time. It is based on the use of a nutrient medium containing sodium bicarbonate as a source of carbon dioxide and of a glass plate imbedded in agar in the Petri dish. The method has been tested with eight spore preparations of *C. botulinum*, Types A and B, and with Putrefactive Anaerobe NCA 3679. In each case the inclusion of bicarbonate in the medium improved the rapidity and completeness of germination. In four cases where the colony count had reached essentially a maximum in 24 hours in the presence of bicarbonate, only from 2 to 12 percent of the spores had formed visible colonies in its absence. Three spore suspensions calibrated at other laboratories and by other methods were counted by the new procedure and were found to contain from 2 to 4.5 times as many viable spores as had been reported.

At least one large commercial research laboratory is now using this counting method not only for *C. botulinum* and Putrefactive Anaerobe 3679, but also for thermophilic and butyric-acid anaerobes. The method thus appears likely to find application beyond that for which it was originally developed.

Water in Fresh and Processed Foods Measured Quickly

Although a number of methods for measuring the water content of various fruits and vegetables have been developed, there remains a need for rapid, reproducible methods employing inexpensive equipment. In particular vacuum-oven methods, which are widely used and form the basis of moisture-specification testing for large-volume food procurement by the Armed Forces, are too slow for control of manufacturing processes in which the product must meet moisture specifications. A new approach to moisture determination has been made under an RMA project at the Western Regional Research Laboratory in the development of a simple, rapid, reproducible procedure in which the nonaqueous substance in a sample is measured by the quantity of potassium dichromate required to oxidize it. The water content of the sample is determined by difference. The method is particularly suitable for materials of high moisture content, such as fresh fruits and vegetables, but may also prove useful for dehydrated products.

The procedure is simple. A measured volume of standard dichromate solution is added to the suitably prepared sample. Concentrated sulfuric acid is added, and the heat of dilution provides instantaneous reproducible heating. Excess dichromate is then titrated electrometrically with ferrous ammonium sulfate solution. An inexpensive circuit of a microammeter, dry cells, and radio potentiometer can be used for this titration, or either a pH meter or commercial titrimeter may be used. The sample need be only coarsely ground or slurried. Depending on the nature of the material, from 5 to 12 minutes are required for a single determination exclusive of the time required for sample preparation. With some samples, about 12 determinations can be made per hour. The quantity of dichromate required for oxidation of the sample is converted to weight of nonaqueous substance by a factor determined for each material by using a reference method for determining moisture. The difference between weight of sample and weight of nonaqueous substance in it represents

the moisture content on the scale established by the reference method.

Although the factor for a given material may be expected to show variations with variety, maturity, and cultural conditions, use of an average value for the factor has been found to result in only moderate errors in moisture content. Thus mean errors of less than 0.5 percent in moisture content were found with widely different samples of dehydrated potatoes, dried prunes, and fresh peas when an average factor for each substance, based on a vacuum-oven reference procedure, was used. Reproducibility of results, a consideration even more important than their accuracy for some applications, is better than 0.2 percent for each of the materials mentioned. The widest difference in factors for such different materials as potatoes, prunes, peas, carrots, and a pudding consisting of rice, pineapple, milk, sugar, and eggs is 6 percent.

TANNIN-BEARING PLANTS

Barks, Leaves, and Roots Studied as Sources of Tannin

Investigations at the Eastern Regional Laboratory on the development of additional sources of vegetable tannins included cooperative studies with the Michigan College of Mining and Technology on the salvage and utilization of waste hemlock bark from lumbering and pulping operations and with the Tennessee Valley Authority on the salvage of oak bark from slabs, completion of the studies and publication of the report on the tannin content of two Florida scrub oak barks, and continued cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering on the development of domestic sumacs, in which more than 3,000 pounds of dried sumac leaf from plants grown at Beltsville, Md., is being evaluated for tannin content and tanning properties, both as ground leaf and as tanning extract.

Emergency production of tanning extracts from canaigre roots feasible

Work on the development of domestic tannins was directed toward expansion of the production and processing of canaigre roots. The investigation was conducted as a cooperative RMA project, in which the Bureau of Plant Industry, Soils, and Agricultural Engineering carried on the field and production work while the Eastern Laboratory evaluated the canaigre roots and studied processing methods.

More than 90 tons of fresh canaigre roots were harvested from plantings at Yuma, Ariz., in 1950. Of these, about 20 tons were shipped to the Eastern Laboratory for processing tests, 5 tons were used in a field-storage test, and the rest were shredded, air-dried, and stored for future processing into tanning extract. Canaigre roots collected from native stands in six of the Southwestern States varied considerably in tannin content. The strains highest in tannin, ranging from 31.3 to 41.9 percent (on moisture-free basis) were found in Arizona, Nevada, California, and Utah. Storage of canaigre roots, pending their processing into tanning extract, is important to permit all-year use of equipment. Preliminary storage tests were conducted, but further study is needed to develop a successful procedure.

Preliminary fermentation studies at the Eastern Laboratory indicated that the purity of canaigre tanning liquors, obtained by a continuous extraction process, could be materially improved by employ-

ing a continuous fermentation process using *Aerobacter aerogenes* for the removal of sugars. Further work on this method is necessary to determine the processing details required to prevent loss of tannin. The use of this continuous process reduced fermentation time from 16 hours to 4 hours.

By using an extraction temperature of at least 150° F., the liquors are effectively pasteurized. This reduces the cost of the extraction process by eliminating an additional pasteurizing step requiring heating and subsequent cooling of the liquors.

The starch in canaigre roots interferes with hot-water extraction of the tannin. Removal or destruction of the starch without adversely affecting the tannin would increase the efficiency of tannin extraction with hot water and might make possible the use of conventional leaching equipment. Certain strains of *Aspergillus niger* were found to grow very well when submerged in canaigre liquors, and the tannin was not appreciably attacked in the early stages of the growth. The coarsely shredded, air-dried canaigre roots were inoculated with spores of *A. niger*, after which water was added and slowly circulated over the shreds. Air required for the growth of the fungus was introduced into the stream of water. After the fermentation was completed, the shreds were extracted without difficulty with water at 194° F., and up to 85 percent of the tannin originally present was recovered in the liquor.

Additional laboratory tanning tests have been made with spray-dried canaigre extracts prepared on a small pilot-plant scale. The resulting leathers were of the heavy or sole-leather type and were well tanned, suitably filled, firm, and of good appearance. Their quality indicated that commercial-scale tanning tests with canaigre extracts would be justified when canaigre extract in sufficient quantity becomes available.

A process for producing tanning extract from canaigre roots has been developed, and experiments made thus far appear to have demonstrated the technical feasibility of commercially manufacturing this domestic tanning material. Production cost under normal conditions would be somewhat higher than the cost of competing imported materials, but not unreasonable in emergency times.

HIDES AND LEATHER

Alum Retannage of Insole Leathers Improved and Simplified

Studies on alum retannage of vegetable-tanned insole leathers to make them more durable, at the Eastern Regional Laboratory, showed that commercially available basic aluminum acetate solution could be substituted advantageously for the mixture of aluminum sulfate, sodium carbonate, and sodium acetate previously used. This substitution resulted in a reduction of the mineral salts in the leather and simplified the application of the retannage in the tannery.

A further improvement in the retannage method was the development of a dry-dipping process. Dry vegetable-tanned leather is dipped in a basic aluminum acetate solution of proper concentration (usually about 11 percent) for about 30 minutes at 113° F. The leather absorbs the solution containing the aluminum acetate, and

retannage (fixation of the aluminum oxide in the leather) is accomplished while the leather is "sammied" (held in a moist condition) for 48 hours. After this treatment the leather is dried and finished.

In cooperation with a commercial producer of cut insoles, who was interested in improving his product, three alum-retannage tests were conducted. Alum retannage by the dry-dipping procedure was applied directly to cut stock. It was demonstrated that acceptable fixation of aluminum oxide could be accomplished by this procedure. The retanned cut insoles contained aluminum oxide ranging from 1.5 to 3.0 percent. In general, they showed a small loss in area, from 0.2 to 1.2 percent, and a gain in thickness, from 10 to 12.5 percent. The cooperating company thought that some of the insoles might be raised from grade B to grade A as a result of the increased thickness. All the samples retanned in these experiments had acceptable shrink temperatures (above 212° F.) and withstood the 3-minute boiling test without shrinkage. They were, however, too stiff and showed some crackiness at the edges of the soles.

In further studies on the application of the dry-dipping method of retannage to cut insole leathers, 320 soles were cut from 40 commercially tanned bellies and retanned at the Eastern Laboratory. Because the leathers varied in absorptive properties, they took up different quantities of the tanning solution and were thus retanned to different degrees. The cut soles were divided into three groups according to their estimated aluminum oxide content: (1) Up to 2.5 percent; (2) 2.5 to 3.0 percent; and (3) 3.0 percent and above. After wetting back, light reoiling, and reworking to relieve excessive stiffness, 294 of the retanned cut soles were furnished to the Philadelphia Quartermaster's Laboratory for use in exposure tests under various atmospheric conditions.

Application of the dry-dipping method of retannage to finished leather in the form of cut soles has given some undesirable results. These include excessive stiffness of the leather and crackiness of the grain (particularly at the edges of cut soles where there appears to be overtannage), together with a loss of area and some distortion of the cut soles that make necessary recutting to a smaller size. Recent tests indicate that these difficulties in alum retannage can be largely overcome by applying the retannage to rough belly centers instead of to finished cut soles, removing excessive tanning and filling materials by washing before retannage, and light reoiling after retannage. Further studies in this direction are in progress.

The importance of the successful application of alum retannage to military insole leathers is evidenced by the following statement from the Research Director of the Research and Development Branch, Textile, Clothing, and Footwear Section of the Office of the Quartermaster General (quoted from Leather and Shoes for October 28, 1950): "Currently a specification is being prepared based upon a combination vegetable and metallic retannage. Some technical problems still remain to be worked out, but the prospect of our adoption of such a combination tanned insole, with particular emphasis upon alum as a retannage, should be faced by the industry, and further plant trials run voluntarily by individual tanners. * * *"

ANIMAL FATS, DERIVATIVES, AND PRODUCTS

Improved Oleic Acid Produced Commercially From Animal Fats

Oleic acid of an improved technical grade is now being produced commercially from animal fats as the result of a process developed by laboratory and pilot-plant investigations at the Eastern Regional Laboratory. Because of its superior properties, this new chemical is finding increasing acceptance for many important industrial uses, and it is being manufactured in tank-car quantities by several large processors of fats and fat acids.

Oleic acid is the most important single component of animal fats, constituting nearly half of their weight. Previous processes for its manufacture did not remove certain impurities that frequently resulted in the development of gummy substances and undesirable color and odor. These properties, traceable to impurities, have limited the use of oleic acid in a number of potential products. The improved oleic acid, free from undesirable impurities, is already finding important outlets, and its production is expanding the utilization of inedible animal fats substantially. It appears to be particularly promising for use in newer chemical applications, such as the preparation of plastics, plasticizers, and soaps having improved stability.

Certain Fat Derivatives Improve Vinyl-Type Plastics

Investigations on the use of vinyl esters and ethers of long-chain fat acids for use in modifying the properties of vinyl-type plastics have been continued under an RMA project at the Eastern Regional Laboratory with encouraging results. Because of the alteration and improvement of physical properties made possible by including a quantity of such fat monomers in the polymerization recipes, they have continued to attract interest from the plastic industry. The points of special industrial interest include greater water resistance and less tendency toward embrittlement caused by low temperatures and migration of plasticizer.

To provide necessary cost data and sufficient material for more thorough product evaluation, arrangements are being made for an investigation on the preparation of certain vinyl esters and ethers of fat acids under an RMA research contract to run through the next fiscal year.

Synthetic Detergents May Come From Animal Fats

Synthetic detergents prepared from substances other than fats are replacing an amount of soap that, according to reliable estimates, would require about 350,000,000 pounds of animal fats for its manufacture. Synthetic detergent derivatives of animal fats are manufactured and sold, but none has been marketed in significant quantity thus far.

An answer to the problem of providing suitable competitive detergents from animal fats is being sought at the Eastern Regional Laboratory through the experimental preparation and evaluation of detergent products from animal fats. Compounds receiving particular attention are higher alcohols, amides, and certain other fat-acid deriva-

tives of animal fats. Current studies include preparation or synthesis, tests of detergency, methods of compounding and building detergents, and pertinent evaluation data. It is too early to forecast whether all the problems, including those of cost, can be solved, but it has already been established that excellent detergents can be made from animal fats.

Use of Animal Fats in Tin Plating Proposed

Suitable processed animal fats may soon replace palm oil for use in hot-dip tinning by tin-plate manufacturers. Scientists at the Eastern Regional Laboratory have prepared various processed samples from inedible tallow and several grades of lard and inedible grease as starting material. These have been evaluated in the laboratories of the Armour Research Foundation, the work being done under contract in accordance with provisions of the Research and Marketing Act of 1946. The required processing is inexpensive and frequently involves only deodorization and adjustment of the free-fat-acid content to about 8 percent. In some cases a mild refining and hydrogenation treatment may also be desirable.

The laboratory tests have facilitated the selection of a promising material for mill-scale evaluation in the production of tin plate for can manufacture. Success in this research would open a market of at least 15,000,000 pounds per year for the animal fats. Possibly more important is the fact that this development would relieve an important industry of its present complete dependence upon palm oil, which must be imported and stockpiled.

WOOL AND WOOL PRODUCTS

Felting Properties of Wool Improved

In research on chemical derivatives of wool and mohair under an RMA project, the Western Regional Laboratory has applied a chemical treatment to wool that markedly enhances its felting properties.

The ability of the fibers to felt, that is, to become firmly entangled, is a distinguishing and useful property of wool. In the finishing of woolen goods, the fabrics are subjected to felting conditions in the fulling step in order to impart desirable body to the finished material. The felting of wool also forms the basis for an industry that produces annually about 25 million pounds of wool felts, excluding woven felts, having a value of between 20 and 30 million dollars. In the manufacture of felts an average of about 10 pounds of new wool is used per pound of reprocessed wool. Wool felts are used as oil retainers, wicks, sound insulators, vibration-resistant mountings, gaskets, etc. Improvements are desired in felting and fulling of wool and woolens because the traditional processes are slow and costly.

The new chemically modified wool is made by treating wool with propiolactone, a chemical that has recently become available commercially. Laboratory studies of propiolactone-modified wool have shown excellent results in tests of felting. Both the rate and degree of felting were increased. Commercial-scale runs at two felting plants have confirmed the marked improvement in felting properties. Felts prepared from the treated wool, which are soft and pliable when wet, become extremely hard on drying; the resistance to splitting and

tensile strength are significantly higher than for felts made from normal wool. The increase in felting power is also found in blends of the treated wool with commercial felting stocks.

Laboratory tests indicate that wool flannel also felts more readily after being modified with propiolactone, as demonstrated by hand-milling shrinkage tests. In both loose-wool and cloth tests, the felting ability of the modified wool varies with the extent of treatment and passes through a maximum at about 50 to 60 percent uptake of propiolactone; more extensive treatments impart shrink-resistance to flannel cloth. Mohair modified with propiolactone also shows greater felting ability.

A number of other chemical treatments of wool have been investigated to determine their effect on felting, but none has been found to increase the rate or degree of felting. These results seem to indicate the unique character of the propiolactone treatment.

Propiolactone may also prove valuable for the treatment of artificially crimped wool and mohair. Such artificially crimped fibers have improved weaving properties and abrasion resistance, but they suffer from low breaking elongation. The subsequent treatment with propiolactone tends to recover the characteristic high-breaking elongation of the normal fibers without destroying the desirable properties imparted by crimping.

The conditions for treating wool with propiolactone have been investigated with respect to solvent, lactone concentration, prior drying of the wool, pH, fineness of wool, temperature, and other factors. The treatment of air-dry wool with a 3 percent solution of propiolactone in carbon tetrachloride, having a lactone-to-wool ratio of about 3 to 1, appears to be a satisfactory procedure, but additional laboratory investigation is required before the propiolactone treatment can be recommended for commercial adoption.

MILK WASTES AND BYPRODUCTS

Work on Dairy-Waste Disposal Shows New Sources of Vitamin B_{12}

The dilute processing waste from factories handling fluid milk is a serious source of pollution in many streams. Unfortunately, the treatment of such waste by customary sewage practices is not satisfactory because the high oxygen demand of the organic matter favors the ready conversion of lactose to lactic acid, which impedes further microbiological action. Milk wastes are therefore difficult to treat in conventional sewage-treating plants. A study of the aerobic microbiological oxidation of milk wastes at the Eastern Regional Laboratory, under an RMA project, has shed new light on what happens during such a treatment. Two possible new processes have been proposed to the industry on the basis of the knowledge thus obtained.

The essential finding was that the organic substances in milk wastes are assimilated very fast by the micro-organisms that are naturally present. The soluble proteins and sugar are thereby transformed to insoluble microbial cells which collect as sludge. One of the methods considered for utilizing this transformation would require centrifugal separation or filtration to remove the micro-organisms, followed by discharge of the innocuous liquid effluent to a stream. A second, partial treating, process has been proposed for use when the effluent is

to be discharged to a municipal sewage-treating plant or to a stream that does not have to be kept at the highest degree of purity. It consists of aeration for 8 to 16 hours, followed by discharge of the whole effluent, containing the microbial cells, to the stream. The pollution load is thereby reduced about 75 percent. Equipment for this simple fill-and-draw procedure, to be repeated daily, should be inexpensive to install and operate. A trial of this process in an operating dairy plant will be made during the coming year, if satisfactory cooperation can be arranged.

The discovery that a large part of the organic matter in dairy waste could be recovered as bacterial cells or sludge following aerobic oxidation of the waste stimulated an investigation of the possible utility of the sludge. Surprisingly high concentrations of vitamin B_{12} were found to be present by microbiological assay procedures. This discovery suggested the possibility that activated sludge from municipal sewage-treating plants might also contain this vitamin. Activated sludge is a trade name for the flocculent mass of micro-organisms that is maintained in the aeration tanks to oxidize the organic constituents of the waste. Excess of sludge is dried and sold as a fertilizer. More than 100,000 tons of the dried material is marketed annually from the four large municipal plants that produce dried-sludge fertilizer.

Tests of the vitamin content of the commercial dried sludge from these four plants showed that three of the four contained 1.5 to 2 milligrams of vitamin B_{12} per pound. A vitamin-B_{12} content of 1.5 milligrams per pound has been proposed by the Association of American Feed Control Officials as a required level for a commercial feed supplement. To supply the needed concentration of vitamin B_{12} in a ton of mixed feed would require the addition of only 2 pounds of such commercial activated sludge.

Chick-feeding tests, conducted by the Bureau of Animal Industry, have confirmed the presence of vitamin B_{12} in activated sludge. The amount indicated by chick-growth assay was somewhat less than that shown by microbiological assay, but it was still of practical significance. Further chick-feeding tests are under way.

A study of the amount of vitamin B_{12} in the fresh sludge showed large losses during drying. Experimental samples dried under mild conditions contained up to 3 milligrams per pound. If the use of dried sludge in mixed feed is approved by feed-control officials, the losses of the vitamin during drying should be investigated further, for such feed supplements are sold on a vitamin-content basis.

Two Enzymes Separated From Milk

Since 1949 an intensive study of certain enzymes in milk has been under way at the Eastern Regional Laboratory with both immediate and ultimate objectives. The immediate, rather limited, objective is the preparation of the enzymes in pure form for investigating their enzymic (catalytic) properties and their properties as proteins. They are excellent models for structural and other studies of the proteins because of the inherent unique arrangement of the component amino acids that gives the enzymes their highly specific catalytic properties. The ultimate objective of the studies is elucidation of enzymic properties that can make the enzymes useful in medicine or industry.

Two enzymes peroxidase and alkaline phosphatase, have been studied most intensively. Peroxidase belongs to the group of proteins that contain hemin, an iron-porphyrin compound. A solution of the peroxidase, greenish in color, catalyzes the oxidation of various compounds by hydrogen peroxide. A method has been devised for separating this enzyme in crystalline form from skimmed milk. Alkaline phosphatase catalyzes the hydrolysis of phosphate esters into their components, phosphoric acid and an alcohol. It is called alkaline phosphatase because it functions best in alkaline solutions. This enzyme, although not yet obtained in pure form, has been prepared in a purity 5,000 times greater than that of the phosphatase as it exists in milk. However, the best preparation still contains three protein components.

Chromatographic adsorption has been a useful tool in the study of these enzymes. Displacement chromatography on a column of calcium phosphate has provided a key step in the purification of the peroxidase. Purification of the phosphatase was impossible by this means, because, once adsorbed, the phosphatase could not be recovered quantitatively. However, chromatographic adsorption did provide a means for distinguishing two forms of the phosphatase. One form, apparently a fat acid-protein complex, is strongly adsorbed on diatomaceous silica and is difficult to purify. Another form, the free protein, is not adsorbed on diatomaceous silica. The phosphatase has been obtained in this form by dissociating the fat acid-enzyme complex with *n*-butanol, a new reagent for the purpose. It is this form of the enzyme that has been obtained in highly purified form.

Continuation of these studies should be of value in elucidating the structure of the biologically and industrially useful proteins and may suggest uses for these enzymes in medicine and chemical industries.

PINE GUM AND PRODUCTS

Improved Equipment, Practices, and Products Benefit Gum-Turpentine Producers

Continuous distillation of cleaned pine gum

A continuous steam still having a stripping column 32 inches in diameter and a maximum capacity of 5 tons of cleaned pine gum per hour was developed and thoroughly tested by the Naval Stores Research Division for commercial use. The operation of a 12-inch continuous steam still was reported in 1948. The additional work with the 32-inch still was done to achieve the present capacity, which is equal to that of the larger batch stills in the industry and is believed to be the maximum desired by any processor of pine gum. The rosin produced in typical continuous runs with the new still had a satisfactory melting point and was low in volatile oil, and the turpentine had a low acid number. To realize the maximum benefits of continuous distillation with this equipment, commercial plants would operate 24 hours each day. Continuous distillation would result in a 50-percent saving in steam consumption, reduction in labor and other operating costs, high-quality rosin and turpentine, and less variation in product quality. Completion of this new still ends nearly 10 years of process-development research.

Complete engineering data for the still can be furnished, on request, by the Naval Stores Research Division in Olustee, Fla.

Improved practices in gum cleaning

To help processors who use the Department's public-service patents on gum cleaning meet the demand for high-quality products, the Naval Stores Research Division this year supplied them with details on improved practices of gum filtration and washing and the handling of chips.

The filtration of crude pine gum by the gum naval stores industry is a phase of plant operation in which savings in labor, time, and material costs can be of great value. To obtain these improvements the Naval Stores Research Division, in cooperation with a filter manufacturer, modified a commercial self-cleaning filter to adapt it to pine-gum processing. The new-type filter requires only the part-time services of one man for operation, uses more economical filter media, and can handle the low-quality gum that gives trouble in the filters generally used by the industry. The capacity of the filter, comparable to that of the conventional types, is such that only one cleaning per day is normally required. The first commercial installation of the new self-cleaning filter has been made.

The attention of processors was called to the necessity for the thorough washing of gum during cleaning to produce high-quality rosin. Although a single washing in the Olustee process produces high-grade, brilliant rosin, such rosin may still contain traces of contaminating materials. A thoroughly clean rosin can be made by recirculation of the filtered, washed gum a second time through the wash tank.

A third simple step in cleaning operations is recommended to processors as a means of upgrading their pale rosin when there is a price differential in favor of the paler grades. It comprises separating the chip cleanings from the main charge and distilling them with a lower grade of gum. This slight but important change in the regular procedure will insure getting the best grade of rosin that the charge can yield. If the chip cleanings were distilled separately they would yield rosin that is several grades lower than the grade of rosin yielded by the gum from which the chips were separated.

Peroxides from turpentine

The production of peroxides from turpentine and their use as replacements for the peroxide catalysts generally used in making synthetic rubber have been reported previously. This development has defense significance because the usual catalysts are based on benzene, which is in short supply. On the other hand, turpentine is abundant. Out of the 271,880 barrels of gum turpentine produced last season, only a small part went into industrial uses. Replacement of the benzene derivatives by terpene peroxides in the manufacture of about 700,000 tons of synthetic rubber would consume about 50,000 barrels of turpentine. This quantity of turpentine would substantially increase the proportion going into industrial uses.

In laboratory-scale tests, the Akron laboratories of the Office of Rubber Reserve last year demonstrated the superiority of the terpene peroxides, such as pinane hydroperoxide and menthane hydroperoxide, over cumene hydroperoxide, which was obtained from benzene. These

laboratories recently confirmed the superiority of pinane hydroperoxide on a pilot-plant scale. They prepared and tested approximately 3,000 pounds of "cold" rubber (synthetic rubber made at relatively low temperature), using pinane hydroperoxide produced by the Naval Stores Research Division. The reaction rates and maximum conversion obtained with pinane hydroperoxide were considerably higher than those realized with the same quantity of cumene hydroperoxide at each of the levels investigated. The properties of the rubber made with terpene hydroperoxide as the catalyst were equivalent to those of similar stocks made with cumene hydroperoxide. Commercial production and use of the terpene peroxide, which appears feasible, would have the advantage, under current conditions, of conserving benzene supplies.

NATURAL RUBBER SOURCES

Guayule Shrub Promising as Domestic Source of Rubber

Investigations directed toward the development of new or improved processes for the extraction of rubber from guayule shrub—a potential source of domestic natural rubber—were continued in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering at the U. S. Natural Rubber Research Station, Salinas, Calif. The investigations are part of a broad program of preparedness against loss of supplies of natural rubber from the Far East and are authorized by the Critical Materials Stock Piling Act of July 23, 1946.

Milling of guayule shrub made continuous

A basic improvement in the time-honored pebble-milling process for recovering rubber from guayule was described last year. Briefly, it consists of processing the freshly harvested shrub rather than shrub that has been conditioned, that is, subjected to prolonged storage or to both field exposure and prolonged storage. Conditioning was formerly thought to be necessary for effecting complete coagulation of the rubber so it could be removed by pebble milling. As pointed out in last year's report, the development of means for processing the shrub immediately after being harvested minimizes or prevents degradation of rubber from exposure to sun in the field or from prolonged storage before milling. That process is believed to simplify and lower the cost of shrub handling, to result in higher over-all yields, and to yield a crude rubber of greater uniformity and superior quality.

Further improvement, with particular reference to the pebble-milling operation, has subsequently been demonstrated through the installation and operation of a continuous tube-pebble-milling circuit in the pilot plant. This installation embodies a number of significant advances over the conventional process as used commercially and by the emergency rubber project during World War II.

The present milling circuit includes a controlled-feed mechanism for introducing crushed guayule shrub at a predetermined rate into the tube mills, improved rectangular flotation tanks provided with means for continuous removal of plant waste from the bottom, and an automatic device for removing cork fragments from the rubber agglomerates, commonly called worms. Two tube mills, 27 inches in diameter by 12 feet long, are used in series for the milling proper, but two Jordan engines (commonly used in paper mills to refine pulp)

will be used to investigate the possibility of replacing one or both tube mills with a more efficient milling device.

Guayule-rubber factories have been characterized by high labor requirements. However, the present pilot-plant installation represents not only mechanical improvement but also a significant saving of labor at several points. A notable example is the automatic device for removing cork fragments, which eliminates the need for two men formerly required to charge and discharge the batch equipment. This new device, by impregnation under high pressure, effects an almost instantaneous water-logging of the cork fragments that accompany the rubber worms from the primary flotation. Previously this was accomplished by low-pressure treatment that required a 40- to 60-minute soaking, as well as additional labor. Other savings in labor have been effected in connection with shrub treatment before milling and with milling operations in general.

Freshly harvested shrub is being processed experimentally. Throughput of shrub is about 150 pounds per hour on the defoliated dry-weight basis. Extensive millings have been conducted in conjunction with a deresination operation, primarily in order to produce enough rubber for evaluation by rubber manufacturers, especially in the form of experimental truck and passenger-car tires.

Quality of guayule rubber improved by deresination

A combination of processing improvements, including the milling of freshly harvested shrub and a deresination treatment, has yielded a guayule rubber believed to be superior to any produced by previous methods. Crude guayule rubber, as normally recovered by the pebble-milling process, contains about 20 percent resins. This gross impurity limits the usefulness of the rubber and contributes to lack of uniformity and to poor aging qualities in storage. Therefore intensive efforts are being made to develop a practicable process for removal of the resins. There are two aproaches to this problem. One consists of extracting the resins from the comminuted shrub with a resin solvent (acetone) before pebble milling. This yields a crude rubber of very low resin content, provided as much as 65 percent of the total shrub resin has been extracted. The other method comprises extraction of the resinous rubber agglomerates, or worms, obtained in normal milling. Tests of rubber quality indicate that the two procedures can be made to yield deresinated rubbers that are essentially equivalent in physical properties.

A batch method was developed and applied on a small pilot-plant scale for deresinating the crude resinous rubber worms as recovered by pebble milling. This procedure is currently considered to have a processing advantage over deresination of shrub, because a much smaller weight of material is processed. Another advantage is that the deresinated rubber worms can be dried more readily and at lower temperature because they are wet only with acetone, a readily volatile solvent. In shrub deresination, the final product is normally wet with water, as the acetone extraction takes place before pebble milling and flotation. High drying temperatures, such as those required for evaporating water effectively, have been found to reduce the quality of rubber. However, the rubber worms present more difficult problems of handling in the solvent-extraction system, and these must be

solved before a satisfactory deresination process can be developed for factory operation.

The present method of worm deresination consists essentially of placing the wet masses of resinous rubber crumbs in a wire-cloth basket supported in a vessel to which acetone is added in the proportion of about 3 to 1, based on the dry weight of the rubber. The individual worms are dispersed into a slurry within the basket, and extraction is facilitated by constant stirring. After 3 to 5 minutes the miscella is withdrawn, and the extraction is continued by adding successive portions of fresh acetone or of partially saturated miscella from a previous run until the residual resin content is reduced to a minimum, usually 1.25 to 2.0 percent. The early part of the extraction is primarily concerned with removal of water from the wet rubber by the acetone, after which resins are progressively solubilized. A hot extraction was found slightly more rapid than a cold one, but a comparison of rate and completeness of extraction for runs made at 30° C. with those for runs made at 50° C. showed no practical advantage for the higher temperature.

Batches of about 35 pounds of rubber worms are deresinated in the small pilot-plant equipment. The extraction system comprises a total of 16 separate leaches with acetone and miscellae that are used in a countercurrent fashion as for extracting oil seeds. Antioxidant in acetone solution is applied after deresination is complete, after which the rubber is spread out on trays for drying.

More than 4,000 pounds of deresinated rubber has already been produced and distributed to different rubber companies. Tires made from it will be evaluated by the Office of Rubber Reserve's test fleet. The purpose of the service tests is to determine whether deresinated guayule rubber is of high enough quality to supplement or replace Hevea rubber in critical uses, such as in the carcass of a heavy-duty truck tire.

THERAPEUTIC AND OTHER BIOLOGICALLY ACTIVE SUBSTANCES

Plant Steroids Studied as Cortisone Precursors

The dramatic announcement of the therapeutic effects of cortisone was made late in 1949. Physicians have found that rheumatoid arthritis, rheumatic fever, severe burns, and a whole group of vague mental and degenerative diseases can be alleviated by this drug. It is estimated that in the United States alone between 5 and 10 million people are suffering from illnesses that can be alleviated by the use of cortisone.

Unfortunately, at present, cortisone is made mostly from the bile acids of cattle by a long and complicated process. Moreover, there are not enough cattle in the world to supply the present and potential demand for the drug. It is thus imperative that a plant source of cortisone precursors be found, so that a crop can be grown in any required amount just for the purpose of supplying the raw material for this drug. It is known that some plants contain small amounts of steroids that have a chemical structure suitable for conversion to cortisone.

The Bureau joined two other groups in a search for these plant steroids which was financed by special funds appropriated to the Public Health Service. Plant specimens from all over the world are being collected by botanists of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Division of Plant Exploration and Introduction. Chemists of the Eastern Regional Research Laboratory are investigating these plants for the presence of possible cortisone precursors. When such compounds are found, they are prepared in substantial quantity and sent to the National Institutes of Health to be converted to cortisone or similar products and tested physiologically.

At present one large order of the vegetable kingdom, the Liliales, is under investigation. This order is known to contain plants in which steroids are found. More than 900 samples, consisting primarily of Yucca, Agave, and Dioscorea species, have been received. The plants for the most part come from southeastern and southwestern United States, Mexico, Central America, and South Africa.

On reaching the Eastern Regional Laboratory the samples are given several preliminary screening tests. As the steroids in plants are combined as saponins, and as saponins dissolve red blood corpuscles, a sample of the incoming plants is first extracted with alcohol, and the extract is used in the hemolysis test. If the result is negative, the plant is discarded. If it is positive, the extract is put through a complete process of isolation on a rapid, micro scale, culminating in a crude steroidal sapogenin preparation. The final product is analyzed by means of infrared absorption. As all sapogenins have characteristic infrared spectra, the sapogenin content of the preparation can be calculated. In this manner many plant samples that have little or no sapogenin content can be rapidly eliminated.

The samples that survive the preliminary screening tests are subjected to large-scale extraction. The saponins are extracted from the plant material with hot aqueous alcohol, and the fats and pigments are removed from the extract with benzene. The saponins are dissolved from the aqueous extract with butanol, leaving behind many interfering substances. The saponins are then hydrolyzed with acid to the steroidal sapogenins. These sapogenins are purified by chromatographic adsorption and are characterized by their chemical properties and by X-ray, ultraviolet, and infrared data.

To date four Agave species from southwestern United States have been found which contain as much as 0.5 percent hecogenin on the moisture-free basis. It is virtually certain that cortisone can be synthesized from hecogenin. Therefore intensive efforts are being made to obtain sufficient quantities of hecogenin for this purpose. The Agave species, and others that may be found later, will be studied from all aspects in order to ascertain whether they can be used as a crop to supply cortisone precursors.

Phosphate Insecticides Inactivate Vitally Necessary Enzymes

The Enzyme Research Division has continued its study, mentioned in the 1949 report, of the reaction between certain esters of phosphoric acid and vitally necessary enzymes in animal and plant tissues. Some of the esters in question are potential war gases; therefore more information about them from the standpoint of protection is desirable.

Other esters of this type are rapidly finding application as insecticides. Many of them are uncommonly poisonous and should not be used in ignorance. Further knowledge of their effects in both plants and animals is urgently needed.

The discovery of a definite chemical reaction between one of these toxic substances (diisopropyl fluorophosphate) and an important enzyme of the animal body (chymotrypsin) has been previously described. By this reaction the enzyme becomes entirely inactive.

It now appears that the same chemical reaction occurs between chymotrypsin and a number of phosphate esters other than diisopropyl fluorophosphate but analogous to it and possessing toxic and insecticidal properties. The substances selected for testing represented various types of these phosphate esters. In every case the enzyme took up only one phosphate group per molecule of enzyme (which is the reaction previously described); yet that very small change was sufficient to inactivate the enzyme completely.

While the reaction was shown to be the same in each case, the speed with which it took place varied very much from one inhibitor to another. The most active inhibitor tested (diisopropyl fluorophosphate) reacted about 1,000 times faster than the slowest one tested. As the reaction is the same in each case, it would appear that those that seem to be most poisonous must react most rapidly. Measurements were made on the following compounds, listed in order of decreasing activity: Diisopropyl fluorophosphate (a potential war gas), tetraethyl pyrophosphate (an insecticide), diphenyl chlorophosphate (a phosphorylating reagent), diethyl thionofluorophosphate, tetraisopropyl pyrophosphate, diethyl *p*-nitrophenyl phosphate, diethyl *p*-nitrophenyl thionophosphate (the insecticide Parathion), tetrapropyl dithionopyrophosphate.

Thus a wide variety of insecticidal phosphates share the property of reacting with chymotrypsin. There is also good reason to believe that the same reaction occurs with other vital enzymes, and underlies the toxicity of the so-called nerve gases. Trypsin was shown by the Enzyme Research Laboratory to undergo exactly the same reaction, while from other laboratories (notably in England) evidence is forthcoming that the important brain enzyme cholin esterase, whose inactivation is promptly fatal, behaves in all probability as do trypsin and chymotrypsin.

Now that the chemical mechanism of the poisoning is known, it is apparent that its reversal would constitute a detoxification, and thus be an important objective for those who are searching for antidotes and protective drugs. Such reversals have not yet been satisfactorily accomplished in the laboratory, although it has been observed that some plants, notably citrus trees, seem to possess a mechanism that reverses the enzyme inactivation in some cases, though not in all.

More Learned About Physiological Action of Rutin

The flavonoids are plant pigments closely related to one another in chemical structure. They are rather widely distributed in various articles of diet, as exemplified by the rutin in asparagus, the quercetin galactoside in the skins of certain varieties of apples, the hesperidin in citrus fruits, and a phlobotanninlike substance in grapes. Investigations by the Pharmacology Laboratory show that a number of

the flavonoids possess the pharmacological properties responsible for so-called vitamin-P activity and that rutin has a sparing action toward vitamin C and adrenalin, one of the hormones produced by the adrenal glands. This sparing action appears to result from the antioxidant properties of rutin which retard the oxidative destruction of vitamin C and adrenalin, thus permitting small amounts of the two compounds to accomplish the physiological results of larger amounts.

The sparing action toward vitamin C was demonstrated by showing that scorbutic guinea pigs receiving rutin together with subminimal amounts of vitamin C survived significantly longer and showed fewer fresh hemorrhages than animals given the same amount of vitamin C alone. Investigations at McGill University have confirmed the work of the Pharmacology Laboratory. They showed that administration of rutin permitted a 50-percent increase in the relative potency of known amounts of vitamin C given to scorbutic guinea pigs, as judged by changes of the incisor teeth.

These observations on the pharmacological properties of the flavonoids in general and rutin in particular are of practical importance for several reasons. Attention was first focused on the possible nutritional and physiological importance of the flavonoids in 1936 by the report that a beneficial effect on vascular permeability could be demonstrated experimentally in guinea pigs. Perhaps the most frequently stated physiological effect of rutin concerns its clinical effectiveness in patients who exhibit symptoms of capillary fragility and impaired capillary permeability. However, it may be doubted that increased permeability and fragility of the capillaries exist in scurvy, a disease in which such conditions of the capillaries have been supposed to be characteristic. Recent studies by two groups of investigators have shown that the capillaries of scorbutic guinea pigs do not exhibit increased permeability. The demonstrated sparing action of rutin toward vitamin C and adrenalin affords an alternate and satisfactory explanation. The responsiveness to adrenalin of the smooth muscular structures controlling blood flow in the capillaries tends to be maintained, and the decreased oxidative destruction of circulating adrenalin permits a more persistent constrictor action of adrenalin on these muscular structures. The net result is a decrease in tendency to capillary bleeding. The concept of antioxidant activity as a basis for mechanism of rutin action provides a more rational basis for the therapeutic use of rutin in conditions where abnormalities of the capillaries need not be visualized, as in hemophilia, idiopathic methemoglobinemia, and arthritis, where a supplemental action to vitamin C may be desirable. Finally, the antioxidant action of rutin and related compounds, together with their known effects on various enzymes, suggests the importance of the flavonoids as a dietary factor that helps to regulate physiological processes.

Basic Research on Allergens of Agricultural Products Continued

Investigations on the allergens of agricultural products, a basic research project previously supported by allotments from a special research fund authorized by the Bankhead-Jones Act of June 29, 1935, were continued under the general appropriation for 1951. The objectives are to correlate the chemical and physical characteristics of allergens with their specific capacity to induce allergic reactions and

to learn the mechanism of the allergic response in the human body and in experimental animals.

Previous annual reports of this Bureau have related the progressive fractionation and purification of allergenic components of several oilseeds. In each case the active substance, a complex compound of protein and polysaccharide, appeared in the 1A fraction. It was further shown that the allergenic activity resided essentially in the polysaccharide-free protein, belonging to the class of proteins known as natural proteoses.

Cottonseed allergen

An apparently homogeneous, fast-moving component was observed during an electrophoretic study of a fraction obtained from cottonseed allergens, CS–1A. Separation of this fast-moving component and a slow-moving one yielded enough material for cutaneous tests on cottonseed-sensitive subjects. The fast-moving component was the more potent. This fractionation is being continued with the aim of isolating a homogeneous allergen in quantity sufficient to develop solubility data essential for tests of homogeneity.

Enzyme action on allergens

A study of the effects of proteolytic enzymes on the natural proteoses of oilseeds was initiated this year to determine whether their allergenic properties result from marked difference from other proteins in susceptibility to enzymatic hydrolysis.

Limitations of available methods for following the splitting of peptide linkages during the enzymatic hydrolysis of proteins prompted trials to find procedures better suited to the study of effect of enzymes on allergens. A simple, rapid, accurate method for the quantitative determination of amino acids and peptides based on spectrophotometric analysis of their copper complexes was devised. The recoveries of 16 amino acids ranged from 98.2 to 103.6 percent, and the over-all deviation from 100 percent recovery was ± 1.2 percent. Histidine behaved atypically. In addition to the amino acids, nine dipeptides and five tripeptides were studied, and the results were interpreted in relation to application of the method to studying the rate of hydrolysis of proteins. Satisfactory application of the method was made in following the hydrolysis of casein. Incidental to this study an ultraviolet absorption peak characteristic of solutions of the copper salts of amino acids and peptides was discovered. Absorption in the ultraviolet spectrum was so intense that a method more than 100 times as sensitive as the one based on measurement of the color of the copper salts of the amino acids appears possible.

Factors affecting anaphylaxis

Studies were continued to correlate antibody production with gross anaphylactic sensitivity and with sensitivity of excised smooth muscle. Incomplete evidence permits the following generalizations: When guinea pigs are sensitized with ovalbumin, gross anaphylactic sensitivity first appears between the eight and eleventh day after sensitization, reaches a maximum on about the fourteenth day, and then decreases precipitously until the twenty-first to twenty-eighth day. Thereafter gross anaphylactic sensitivity increases slightly to a level that is maintained for the balance of the experimental period. Thus there is a definite inverse relationship between antibody concentration

in the blood and gross anaphylactic sensitivity. On the other hand, there is a direct relationship between antibody concentration in the blood and anaphylactic sensitivity of excised smooth muscle tissue. When alum-precipitated ovalbumin was used as a sensitizing agent, gross anapyhlactic sensitivity appeared at about the same time as with plain ovalbumin; the rise to a peak of maximum sensitivity and the abrupt decline in sensitivity appeared also to require about the same time, but the level of sustained sensitivity was much lower than in animals sensitized with plain ovalbumin. In these animals the antibody concentration increased to a maximum and did not diminish during the experimental period. In these animals, also, there was an inverse relationship between antibody concentration and gross sensitivity and a direct relationship between tissue sensitivity and antibody concentration.

Relationship between anaphylaxis and allergy

A group of workers at the department of allergy, Roosevelt Hospital, New York City, reported that immediate urticarial reactions of the Prausnitz-Kustner type may be produced in human skin after passive sensitization with sera from rabbits sensitized to ovalbumin and with sera from guinea pigs sensitized to pollens of grass or ragweed. That evidence implied that experimental animals produce skin-sensitizing antibodies similar to human allergic antibodies, called reagins. In a cooperative study with those workers, sera from guinea pigs sensitized with single or multiple injections of plain ovalbumin and sera from guinea pigs sensitized with a single subcutaneous injection of alum-precipitated ovalbumin all failed to show a capacity to sensitize human skin. However, in one series of antisera from seven guinea pigs that had survived one or two anaphylactic reactions, the sera from three animals showed definite skin-sensitizing capacity in human subjects. There was no significant difference in anaphylactic titer or skin-sensitizing capacity between the sera prepared after the first and after the second challenging injections. No relationship between anaphylactic titer and skin-sensitizing capacity was apparent. It was concluded that human skin sensitization and gross anaphylactic sensitization are induced by different antibodies.

Serological study of milk proteins

The cooperative study of the serological properties of milk proteins initiated last year with the Protein Division of the Eastern Regional Laboratory was continued. Normal β-lactoglobulin and guanidine-treated β-lactoglobulin were compared by serological methods. The result showed that treatment of normal β-lactoglobulin with 5M guanidine, a denaturating agent, had not altered the serological properties of the recovered crystalline protein.

Effect of dodecyl sulfate on serological properties of proteins

During attempts to isolate a homogeneous β-lactoglobulin at the Eastern Laboratory, a crystalline derivative was prepared from β-lactoglobulin containing two equivalents of firmly bound dodecyl sulfate. This derivative of β-lactoglobulin afforded opportunity to study the effect on the antigenic specificity of the protein of introducing a foreign polar compound. For comparison, a similar product was prepared from serum albumin, which yielded a second protein likewise containing two equivalents of dodecyl sulfate. The two prepara-

tions were compared in serological tests with each other and with their native protein counterparts. Chemical combination of dodecyl sulfate did not change the serological specificity of the native proteins. However, the sensitizing activity of serum albumin, normally a weak senitizing antigen, was increased significantly by combination with dodecyl sulfate.

Allergens of Johnin and Tuberculin Studied

Significance of poor growth of cultures used for production of tuberculin was investigated to determine whether a low yield of bacteria was due to limited bacterial multiplication or to lysis of the bacterial cells. Culture filtrates from poor-growth and normal-growth cultures were subjected to fractionation by procedures previously shown to concentrate the tuberculin. Yield of tuberculin from poor-growth culture filtrate was about one-third of the yield from normal-growth cultures. Bovine skin tests of the two tuberculin fractions were more potent than BAI Regular Tuberculin. Thus the low yield of tuberculin from poor-growth cultures was not due to excessive lysis, but was the result of abnormally low rate of bacterial multiplication.

Fractionation studies were continued with both tuberculin and johnin. Countercurrent extraction of tuberculin-culture filtrates with phenol was found to concentrate the tuberculin in the phenol fraction, but loss of active material was large.

Tests of tuberculin and johnin on problem dairy herds demonstrated the advantage of employing tuberculin and johnin in more than one concentration for evaluation of specificity and potency. Failure to confirm results of skin tests performed on experimentally sensitized animals demonstrated the necessity for developing more reliable criteria for evaluating diagnostic specificity and potency of preparations derived by fractionation of culture filtrates.

RESEARCH APPARATUS AND METHODS

New Chromatographic Methods of Analysis Devised

Various chromatographic techniques have been used in different laboratories of the Bureau for analysis of mixtures of closely related organic compounds for which other methods were lacking or were very difficult and time consuming, and new modifications have been devised for special purposes.

For the analysis of complex mixtures of sugars that result from the treatment of carbohydrates with chemicals or from fermentation and enzyme action, the Northern Regional Laboratory has done pioneer work on the development and use of new techniques in paper chromatography, whereby both qualitative and quantitative analyses of such mixtures can be carried out rapidly and accurately. A new technique for multiple development of the paper chromatogram was worked out to improve the separation of sugars in a mixture. Studies of new solvent combinations and the relationships between the structure of carbohydrates and their behavior on the paper chromatograms gave a basis for choosing the best methods for separating different carbohydrates. The results of this work were published in Analytical Chemistry, volume 23, pages 415 to 420 (March 1951).

These new developments in paper chromatography of carbohydrates already have proved indispensable in connection with several impor-

tant problems of the laboratory, including the breakdown of high-molecular-weight dextrans to fractions having molecules of sizes suitable for use as blood-plasma extenders. Numerous outside requests for information on these methods further indicate their importance.

The Bureau's Fruit and Vegetable Chemistry Laboratory at Pasadena, Calif., devised a new microchromatographic technique to isolate and purify the small amounts of terpene flavoring compounds in the juices of citrus fruits, incidental to work on new and improved uses of citrus products under an RMA project. This technique, which has been named the chromatostrip method, is useful, not only in the field of terpene chemistry, but also in other branches of organic chemistry.

The new technique consists in coating glass strips (about one-half by 5 inches) with an adsorbent mixed with either starch or plaster of paris as a binder. The substance to be chromatographed is spotted near one end of the strip and then developed, as in filter-paper chromatography, with the aid of capillary attraction by dipping the end of the strip in a suitable solvent. The solvent is evaporated from the strip, and the various chromatographic zones of the colorless compounds are indicated by spraying with suitable reagents. Compounds that adsorb bromine can be located by spraying with fluorescein solution and exposing to bromine vapors. Compounds that adsorb bromine faster than fluorescein, such as those containing ethylenic-type double bonds, show up as yellow zones on a pink background. Very unreactive compounds can be located by spraying the strips with a concentrated sulfuric-nitric acid mixture and heating to cause charring of the compounds. This particular reagent is useful in making sure that no compound that is indifferent to the usual reagents for finding colorless compounds on chromatographic columns eludes the investigator.

This microchromatographic technique provides a rapid means of checking solvents and adsorbents for use on larger columns. The R_F value (ratio of distance traveled by a spot to the distance traveled by the solvent) can be used to assist in the identification of compounds. A description of the method has been published in Analytical Chemistry, volume 23, pages 420 to 425 (March 1951).

In order to isolate sufficient material for chemical analysis, the chromatographic method was extended to a larger column of novel design. The new column is not encumbered by a retaining glass envelope but is self-supporting. It is formed by mixing an adsorbent with plaster of paris and casting the wetted mixture in the form of a bar, which may have a glass rod in its center to add strength. A special solvent distributor has been designed to apply solvent uniformly to the bar. Development of the chromatogram is accomplished in a glass cylinder; the bar may be removed at any time to check on the chromatographic development, and after being scraped lightly to remove the reagent can be returned to the same or a different solvent for further development. This is in direct contrast to the usual packed chromatographic column, because once the latter is removed from its glass envelope no further development can be undertaken. The chromatobar, as this new column has been named, is described in detail in Analytical Chemistry, volume 23, pages 428 to 430 (March 1951).

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